CHARACTERISATION AND ANTIMICROBIAL ACTIVITY OF POLY(LACTIC ACID)/KENAF BIO-COMPOSITES CONTAINING A NATURAL AGENT

A thesis submitted for the degree of Doctor of Philosophy

by

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Abstract

The use of materials based on poly(lactic acid) (PLA) as alternatives to petroleum-based polymers for a range of applications has increased in recent years. In the case of food packaging in particular, PLA has experienced growth in combination with the use of a wide range of other materials and/or additives derived from natural and renewable resources. However, the initial costs associated with new technologies to produce PLA and/or other bio-based polymers are typically high, so new techniques are required to reduce costs without compromising material properties and biodegradability. Naturally sourced lignocellulose fibres such as kenaf (*Hibiscus cannabinus* L.) are often used as cost-reducing fillers and/or reinforcing agent for biopolymers such as PLA.

This study explores the characteristics and antimicrobial (AM) activity of PLA and PLA/kenaf composites incorporated with thymol, a natural bio-active AM substance/agent. The production of PLA/kenaf composites containing thymol is intended for potential use in AM packaging applications such as rigid and/or flexible packaging and coatings. Composites of PLA incorporated with 5 to 40% w/w kenaf fibre loadings and thymol concentrations ranging from 5 to 30% w/w were prepared and compared with control systems containing either no kenaf or no thymol. Kenaf fibres can be treated by alkalisation to improve compatibility with polymer matrices. It was found that the PLA/kenaf composites containing treated kenaf possessed significantly higher tensile strength and stiffness than composites prepared with untreated kenaf. Micrograph images of the fracture surface revealed better adhesion between the treated kenaf fibres and the matrix, thereby resulting in improved reinforcement of the composite. Moreover, PLA/kenaf composites containing thymol exhibited lower tensile strength than those without thymol, suggesting a possible plasticizing effect in the presence of the additive.

The PLA systems containing treated kenaf were selected as the main formulation with which to systematically investigate the effect of different processing conditions and formulations on mechanical and thermal properties, the interaction and quantification of AM agent, the migration rate of AM agent from the materials as well as the potential AM properties of these systems. A study of PLA/kenaf composites containing 5% w/w thymol demonstrated that the addition of fibre (10 to 40% w/w) to the composites affected the tensile properties of the system more than the incorporation of thymol alone. Thermogravimetric analyses of PLA and PLA/kenaf composites incorporated with 5% and 10% w/w thymol revealed no significant changes in the decomposition temperature. Analysis by differential scanning calorimetry, however, showed a decrease in all of the key thermal transitions with the addition of thymol. Moreover, the quantification of thymol in PLA and PLA/kenaf composites after thermal processing, as well as the interactions amongst the PLA matrix, kenaf fibres and the AM agent, were investigated. The PLA incorporating a fibre content in the range of 10 to 40% w/w retained less thymol upon processing than PLA alone and the PLA/kenaf composites containing the highest fibre loadings exhibited the lowest thymol retention. The observed losses were attributed to the higher mechanical shear required during the mixing process which may lead to thermal degradation, as well as the creation of voids in the composites that can facilitate the release of thymol from the system.

In order to investigate the migration of thymol from the materials, the PLA and PLA/kenaf films containing 10% w/w thymol were prepared and placed in contact with food simulants comprised of 15% and 95% v/v ethanol/water mixtures at different temperatures. First-order kinetics, diffusion modelling and Fick's law modelling were used to describe the release of the AM agent. The release rate of thymol into 95% v/v ethanol/water at different temperatures displayed Fickian behaviour, with diffusion coefficient values between 1 and 100 × 10^{-11} $m^2\,s^{-1}$ and with close to 100% of thymol being released. The release rate of thymol was temperature

dependent and was affected by the percentage of ethanol in the simulant. In the case of PLA and PLA/kenaf films, a faster release occurred in 95% v/v ethanol/water than in 15% v/v ethanol/water with the composite film exhibiting a higher diffusion coefficient in each case.

The AM activity and stability under different storage conditions for the PLA-based materials containing kenaf fibres and thymol were also investigated. Microbial reductions of 6.8 log CFU mL^{-1} in tryptone soy broth after two days at 37°C and 3.1 log CFU cm^{-2} on processed meat samples after 30 days at 10°C were observed in PLA film containing 30% w/w kenaf and 10% w/w thymol. The PLA/kenaf films containing 20 to 30% w/w thymol showed significant inhibition of microbial growth in solid and liquid media compared to active PLA. The composite films also inactivated *Escherichia coli (E. coli)* that was inoculated on the surface of meat samples. The PLA/kenaf films containing thymol that were wrapped with aluminium foil were able to retain the additive in the films after 3 months of storage at ambient temperature, however films that were not wrapped in foil lost some thymol to the atmosphere. Therefore, the development of active PLA composite films containing the natural fibre as a filler demonstrates a strong potential for the development of active packaging films that can extend the shelf life of certain foods.

Declaration

"I, Intan Syafinaz Mohamed Amin Tawakkal, declare that the PhD thesis by Publication entitled **Characterisation and Antimicrobial Activity of Poly(lactic acid)/Kenaf Bio-composites Containing a Natural Agent** is no more than 100,000 words in length including quotes and exclusive of tables, figures, appendices, bibliography, references and footnotes. This thesis contains no material that has been submitted previously, in whole or in part, for the award of any other academic degree or diploma. Except where otherwise indicated, this thesis is my own work".

[redacted]

Intan Syafinaz Mohamed Amin Tawakkal Date: 26 September 2016

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Publications from this Work

Papers in Refereed Journals

Tawakkal, I. S. M. A., Cran, M. J., Miltz, J. and Bigger, S. W. (2014). A Review of Poly(Lactic Acid)-Based Materials for Antimicrobial Packaging. *Journal of Food Science,* **79**(8), 1477-1490.

Tawakkal, I. S. M. A., Cran, M. J. and Bigger, S. W. (2014). Effect of Kenaf Fibre Loading and Thymol Concentration on the Mechanical and Thermal Properties of PLA/Kenaf/Thymol Composites. *Industrial Crops and Products,* **61**, 74-83.

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Tawakkal, I. S. M. A., Cran, M. J. and Bigger, S. W. (2016). Interaction and Quantification of Thymol in Active PLA-Based Materials Containing Natural Fibers. *Journal of Applied Polymer Science,* **132**, 42160 (1 of 11).

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Tawakkal, I. S. M. A., Cran, M. J. and Bigger, S. W. (2016). Effect of Poly(Lactic Acid)/Kenaf Composites Incorporated with Thymol on the Antimicrobial Activity of Processed Meat. *Journal of Food Processing and Preservation* (accepted for publication).

Conference/Symposium/Colloquium Presentations

Intan S. M. A. Tawakkal, Marlene J. Cran and Stephen W. Bigger, *Development of Active Poly(Lactic Acid) Composites Reinforced with Natural Fibres*, paper presented at the Malaysian Postgraduate Colloquium, Melbourne, Australia, 19-20 December 2015.

Intan S. M. A. Tawakkal, Marlene J. Cran and Stephen W. Bigger, *Development of Active Poly(Lactic Acid) Composites Reinforced with Natural Fibres*, poster presented at the 27th IAPRI Symposium on Packaging, Valencia, Spain, 8-11 June 2015.

Intan S. M. A. Tawakkal, Marlene J. Cran, and Stephen W. Bigger, *Antimicrobial Activity of Poly(Lactic Acid)/Kenaf Composites Containing a Natural Agent*, poster presented at the 5th AIFST Food Science Summer School, Melbourne, Australia, 28-30 January 2015.

Intan S. M. A. Tawakkal, Marlene J. Cran, and Stephen W. Bigger, *Characteristics of Active Natural Fibre Reinforced Poly(Lactic Acid) Composites*, paper presented at the 19th IAPRI World Conference on Packaging, Melbourne, Australia, 15-19 June 2014.

Stephen W. Bigger, Marlene J. Cran and Intan S. M. A. Tawakkal, *Developments in Antimicrobial Food Packaging Research*, paper presented at the 19th IAPRI World Conference on Packaging, Melbourne, Australia, 15-19 June 2014.

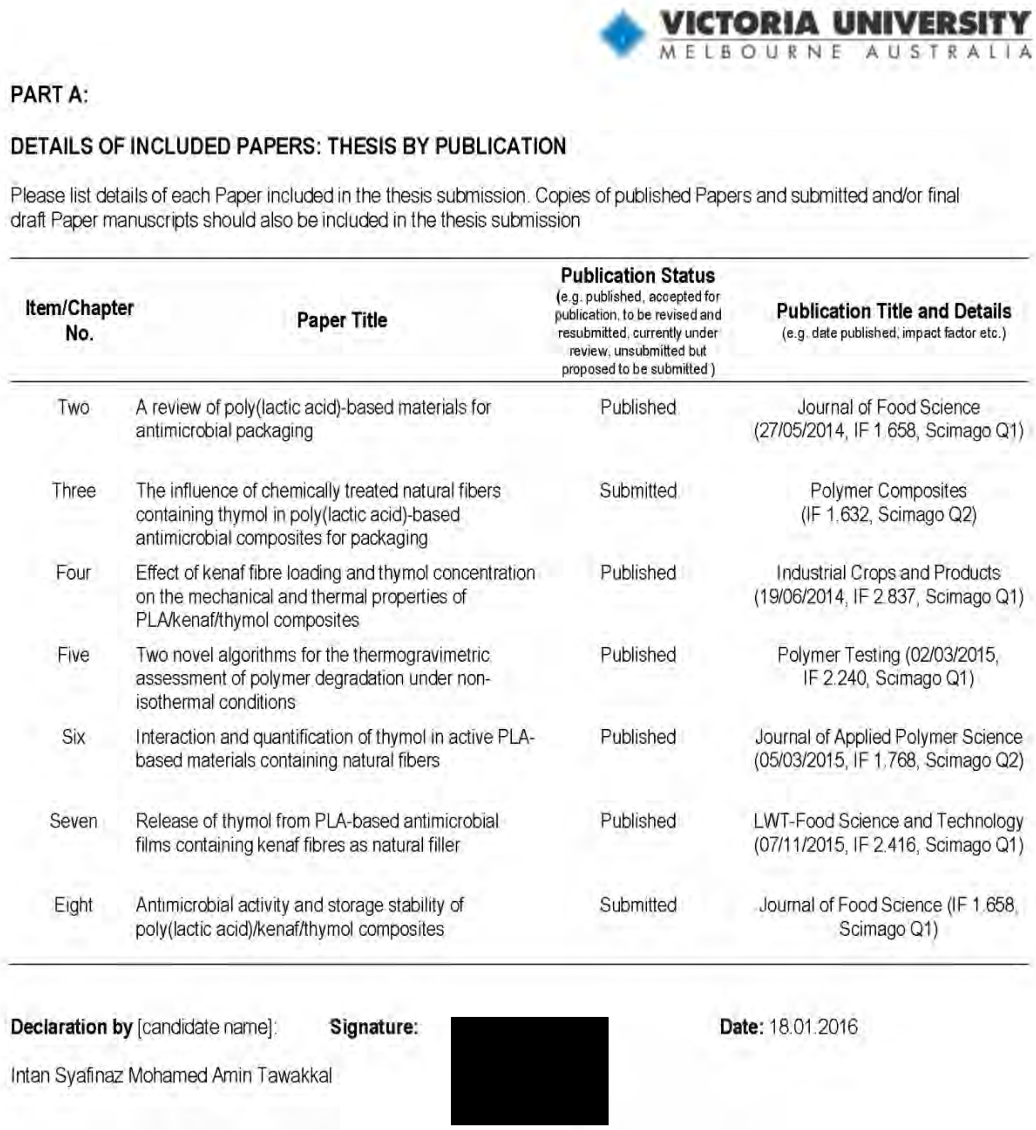
Stephen W. Bigger, Marlene J. Cran and Intan S. M. A. Tawakkal, *Thermal Measurements in Assessing the Performance of Poly(lactic acid)/Natural Fibre Composites for Food Packaging Applications*, paper presented at the 248th ACS National Meeting & Exposition, California, USA, 10-14 August 2014.

Awards and Achievements

Won the Best Presenter Award in the Malaysian Postgraduate Colloquium, Melbourne, Australia, 19-20 December 2015 with presentation entitled *Development of Active Poly(Lactic Acid) Composite Reinforced Natural Fibres,* Melbourne, Australia, 19-20 December 2015.

Winner of the 2nd runners up place in the Victoria University Three Minute Thesis Competition with presentation entitled *Love Food and Nature…Hate waste!*, Melbourne, Australia, 27 September 2013.

Details of Included Papers: Thesis by Publication



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PART A:

DETAILS OF INCLUDED PAPERS: THESIS BY PUBLICATION

Please list details of each Paper included in the thesis submission. Copies of published Papers and submitted and/or final draft Paper manuscripts should also be included in the thesis submission

Item/Chapter No.	**Paper Title**	**Publication Status** (e.g. published, accepted for publication, to be revised and resubmitted, currently under review, unsubmitted but proposed to be submitted)	**Publication Title and Details** (e.g. date published, impact factor etc.)
Two	A review of poly(lactic acid)-based materials for antimicrobial packaging	Published	Journal of Food Science (27/05/2014, IF 1.658, Scimago Q1)
Three	The influence of chemically treated natural fibers containing thymol in poly(lactic acid)-based antimicrobial composites for packaging	Submitted	Polymer Composites (IF 1.632, Scimago Q2)
Four	Effect of kenaf fibre loading and thymol concentration on the mechanical and thermal properties of PLA/kenaf/thymol composites	Published	Industrial Crops and Products (19/06/2014, IF 2.837, Scimago Q1)
Five	Two novel algorithms for the thermogravimetric assessment of polymer degradation under non-isothermal conditions	Published	Polymer Testing (02/03/2015, IF 2.240, Scimago Q1)
Six	Interaction and quantification of thymol in active PLA-based materials containing natural fibers	Published	Journal of Applied Polymer Science (05/03/2015, IF 1.768, Scimago Q2)
Seven	Release of thymol from PLA-based antimicrobial films containing kenaf fibres as natural filler	Published	LWT-Food Science and Technology (07/11/2015, IF 2.416, Scimago Q1)
Eight	Antimicrobial activity and storage stability of poly(lactic acid)/kenaf/thymol composites	Submitted	Journal of Food Science (IF 1.658, Scimago Q1)

Declaration by [candidate name]: **Signature:** [redacted] **Date:** 18.01.2016

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Included papers update:

- Chapter 3 is now published in Polymer Composites.
- Chapter 8 is accepted for publication in Journal of Food Processing and Preservation.

Table of Contents

List of Unpublished Figures

List of Unpublished Tables

List of Abbreviation and Nomenclature

AIT	Allyl isothiocyanate
AM	Antimicrobial
ANOVA	Analysis of variance
AOX	Antioxidant
AP	Active packaging
ASTM	American society for testing and materials
ATCC	American type culture collection
ATR	Attenuated total reflectance
BHI	Brain-heart infusion
BHT	Butylated hydroxytoluene
CFU	Colony forming units
DMA	Dynamic mechanical analysis
DSC	Differential scanning calorimetry
DTA	Differential thermal analysis
E. coli	*Escherichia coli*
EDTA	Ethylenediaminetetraacetic acid
EOs	Essential oils
EVA	Ethylene vinyl acetate
EVOH	Ethylene vinyl alcohol
FDA	Food and drug administration
FID	Flame ionization detector
FTIR	Fourier transform infrared
GC	Gas chromatography

GRAS	Generally recognized as safe
GTA	Glycerol triacetate
JPEG	Joint photographic experts group
LAE	Lauric arginate
LDPE	Low-density polyethylene
LLDPE	Liner low-density polyethylene
L/P	Length to diameter aspect ratio
MC	Methylcellulose
MCC	Microcrystalline cellulose
MFC	Microfibrillated cellulose
MIC	Minimum inhibitory concentration
OLE	Olive leaf extract
PBS	Poly(butylene succinate)
PCL	Poly(ε-caprolactone)
PE	Polyethylene
PEG	Polyethylene glycol
PET	Poly(ethylene terephthalate)
PHB	Polyhydroxybutyrate
PLA	Poly(lactic acid)
PP	Polypropylene
PS	Polystyrene
PVC	Polyvinyl chloride
PVOH	Polyvinyl alcohol
RH	Relative humidity
SAS	Statistical analysis software
SB	Sodium benzoate
SEM	Scanning electron microscopy
SPI	Soy protein isolate
SSE	Sum of squared errors
TBHQ	Tertbutylhydroquinone
TG	Thermogravimetric

TK	Treated kenaf
TS	Tensile strength
TSB	Tryptone Soy Broth
TSBP	Thermoplastic sugar beet pulp
UK	Untreated kenaf
D	Diffusion coefficient
E	Young's modulus
E_a	Activation energy of diffusion
ε	Elongation at break
f	Percentage of fungal coverage
ΔH_{cc}	Enthalpy of cold crystallisation
ΔH_m	Enthalpy of melting
k_1	Diffusion rate constant
k_2	Kinetic rate constant
l	Film thickness
m_t	Amount of AM agent released from the film
m_∞	Equilibrium amount of AM agent released from the film
μ	Specific death rate
N	Population surviving
N_0	Initial population
R	Ideal gas constant
r^2	Correlation coefficient
T	Temperature
t	Time
T_{cc}	Cold crystallisation temperature
T_g	Glass transition temperature
T_m	Melting temperature
v_0	Initial rate of release of the AM agent
$\%X_c$	Percentage of crystallinity

Chapter 1 – Introduction

This chapter presents an overview of antimicrobial packaging with a focus on materials based on poly(lactic acid) (PLA) in combination with natural bio-active agents and plant-based fillers. The overall aims and the thesis outline are also presented in this chapter.

1.1 Overview

1.1.1 Antimicrobial Packaging Systems

Traditional food packaging materials and technologies are designed to provide a physical barrier to passively protect food products from physical, chemical and biological contamination that can lead to the deterioration of the flavour, odour, colour, sensory and textural properties of foods (Risch, 2009). In addition to these functions, more recently developed active packaging (AP) systems are designed to specifically interact with the biochemical or chemical processes in the headspaces or on the surface of food products in order to maintain the safety and quality of the produce, and ultimately, reduce food wastage (Muriel-Galet *et al.*, 2013; Qin *et al.*, 2015a; Ramos *et al.*, 2013). Antimicrobial (AM) packaging, a form of AP, has recently gained attention in the research and industrial sectors with the incorporation of bio-based materials and natural bio-active agents/substances to impart AM activity. Antimicrobial packaging offers benefits over traditional food preservatives including the provision of a continuous AM effect on foods for extended times and the minimization of interactions with and possible inactivation of AM agents by food components (Muriel-Galet *et al.*, 2012).

In AM packaging systems the packaging material, in the form of films or sheets, is used as a vector for the retention of preservatives. Moreover, bio-based polymers are preferable to petroleum-based polymers such as polyethylene terephthalate (PET) due to environmental and food safety reasons (e.g. migration of hazardous additives into foods), and natural, non-toxic bio-active agents are also preferable due to health concerns and ecological issues (Kim and Lee, 2012; Kuorwel *et al*., 2013; Qin *et al*., 2015b). These two main components of AM packaging system which can be generated or produced from renewable resources and are free from toxicity risks associated with the components themselves or migration of undesirable substances into the food, are thus highly in demand (Jamshidian *et al*., 2010). Natural bio-active agents can be integrated into polymeric materials using several techniques including surface coating of formed articles, immobilizing the agents by chemical grafting, direct addition during processing and using polymers that possess intrinsic AM activity such as chitosan (Fernández-Pan *et al*., 2015; Peretto *et al*., 2014).

There are a number of natural AM bio-active agents such as organic acids, bacteriocins (e.g. nisin and lacticin), essential oils (e.g. thymol, carvacrol and linalool), plant extracts (e.g. olive leaf extract), enzymes (lysozyme and peroxidase), chelating agents (e.g. amino acids), metals (e.g. silver) that have been incorporated into AM packaging materials to provide AM activity. A wide range of essential oil (EO) extracts including thymol have been incorporated directly into packaging materials. Thymol (2-isopropyl-5-methylphenol) is a white crystalline substance with a pleasant aromatic odour that is found in the EO of thyme (*Thymus vulgaris*) and it possesses AM and antioxidant properties for a wide spectrum of microorganisms such as bacteria, mould and yeast (Burt, 2004; Nostro *et al*., 2007). Thymol has the potential to inhibit Gram-positive bacteria and to a lesser extent, Gram-negative bacteria, including the pathogenic strains of *Escherichia coli, Staphylococcus aureus* and *Bacillus cereus* (Burt, 2004; Falcone *et al*., 2005; Petchwattana and Naknaen, 2015). The AM activity of thymol has been attributed to its hydrophobic nature whereby it is most likely to partition in the lipid layer of bacteria cell membranes and mitochondria resulting in the leakage of cell contents (Burt, 2004; Guarda *et al*., 2011).

For food applications in particular, thymol is highly suitable as a food preservative and it is classified by the US Food and Drug Administration as a Generally Recognized as Safe (GRAS) food additive. Recently, the AM activity of thymol against spoilage bacteria isolated from fresh produce has been reported by Zheng *et al.* (2013). According to Burt (2004), the concentration of thymol required to achieve a significant AM effect is *ca.* 0.5-20 μL g^{-1} in food products such as fresh meat, meat products, fish, dairy products, vegetables and fruit. Thymol residues in food must be less than 50 mg kg^{-1} in order to be considered safe for consumption as reported by the World Health Organization (Tao *et al.*, 2014). Nevertheless, there are a number of limitations associated with the use of AM substances extracted from essential oils such as limited water solubility, ease of degradation or chemical reactivity, high volatility, and the potential to alter organoleptic properties of foods when used in high concentrations (Guarda *et al.*, 2011; Rodríguez-Martínez *et al.*, 2016; Tao *et al.*, 2014).

Several polymers including petroleum-based and biodegradable polymers have been investigated for thymol incorporation including starch-based polymers (Kuorwel *et al.*, 2013), zein (Li *et al.*, 2012; Mastromatteo *et al.*, 2009), soy protein isolate (Emiroglu *et al.*, 2010; Hu *et al.*, 2012), pullulan (Gniewosz and Synowiec, 2011), polycaprolactone (Del Nobile *et al.*, 2009; Sanchez-Garcia *et al.*, 2008), PLA (Ramos *et al.*, 2014; Tawakkal *et al.*, 2016; Wu *et al.*, 2014), polybutylene succinate (Petchwattana and Naknaen, 2015), polypropylene (Ramos *et al.*, 2012), low density polyethylene (Cran *et al.*, 2010), and linear low density polyethylene (Torres *et al.*, 2014). Most of the aforementioned studies have concentrated on the mechanical, thermal and water vapour barrier properties, the quantification and the migration rate of AM agent from the polymers as well as the potential AM activity of these systems against targeted microorganisms. Some studies have also focused on ternary systems where the inclusion of various additives, fillers and other polymers have been combined, with the main aim being to improve material performance, enhance AM and/or antioxidant properties, reduce costs, and expand into new applications.

1.1.2 Ternary AM packaging systems

The utilisation of bio-based materials, particularly in food packaging applications such as rigid and flexible sheets and/or films, edible films and coatings, has become popular due to the environmental benefits associated with these being renewable resources and the growing economic pressure to reduce the dependence on fossil resources (Johansson *et al*., 2012). Moreover, consumer preferences for natural food products with few or no preservatives and minimal microbial contamination while using disposable, potentially biodegradable and recyclable packages, has generated a growing interest in the use of bio-based materials in AM packaging. Formulations of PLA containing various types of AM and antioxidant agents have been investigated by a number of researchers (Byun *et al*., 2010; Hwang *et al*., 2013; Jin and Zhang, 2008; Liu *et al*., 2010; Rodríguez-Martínez *et al*., 2016). Of the reported studies, many have investigated the incorporation of non-volatile antioxidants in particular (e.g. resveratrol, α-tocopherol and butylated hydroxytoluene (BHT)) and immobilized AM agents (e.g. nisin and lysozyme) with little attention having been devoted to investigating a ternary system of an antimicrobial PLA containing a bio-filler. One of the reasons may be attributed to there being less focus on the development of bio-composite materials containing natural fibre fillers in packaging applications. Moreover, the initial costs associated with new technologies to produce biopolymers such as PLA are typically high so new techniques must be sought to reduce costs without compromising material properties (Mensitieri *et al*., 2011). For example, naturally-sourced fibres are commonly used as cost-reducing fillers for PLA (Gurunathan *et al*., 2015; Saba *et al*., 2015).

In a recent study, PLA was incorporated with nanocellulosic-fillers derived from plants such as microfibrillated cellulose (MFC), cellulose whiskers or cellulose crystals to produce nanocomposite materials that have advanced, high-performance characteristics (Arjmandi *et al.*, 2016; Herrera *et al.*, 2015). The incorporation of nanocellulosic fillers such as microfibrillated cellulose improves polymer mechanical properties such as tensile strength and modulus as well as water vapour barrier properties in a more efficient manner even at low filler loading than is achieved in conventional micro- and macro-filler composite materials (Arjmandi *et al.*, 2016; Halász and Csóka, 2012; Herrera *et al.*, 2015; Siró and Plackett, 2010). There have been few reports that have investigated PLA-based nanocomposites intended for AM food packaging applications (Abdulkhani *et al.*, 2015; Almasi *et al.*, 2014; Liu *et al.*, 2014; Salmieri *et al.*, 2014). Almasi *et al.* (2014) prepared solvent cast PLA-based nanocomposite films incorporated with modified cellulose nanofibre (MCNF) and tert-butylhydroquinone (TBHQ) antioxidant with the aim of determining the efficacy of the antioxidant films to prolong the shelf-life of fatty foods. They reported that the release rate of TBHQ in soybean oil was significantly decreased by the presence of MCNF, which was enough to delay the induction of the oxidation of soybean oil stored for 6 months. Nevertheless, the mechanical separation of plant fibres into smaller elementary constituents typically requires high energy input and the lack of compatibility with hydrophobic polymers can result in significant aggregation of these nanosized fillers (Dufresne, 2013; Herrera *et al.*, 2015; Oksman *et al.*, 2015). Moreover, possible migration of nanosubstances can potentially have an adverse impact on human health (Abdul Khalil *et al.*, 2012; Bumbudsanpharoke and Ko, 2015).

Natural lignocellulose fibres derived from a variety of plants such as wood, kenaf, jute, flax, ramie, hemp, pineapple leaf and bamboo could be used as reinforcements in biopolymers to generate composite materials with opportunities in biodegradable packing applications. Natural fibres possess advantages such as low density, relatively high specific strength and stiffness and impart less equipment abrasion (Awal *et al*., 2015; Wambua *et al*., 2003). These advantages have also initiated much attention in replacing inorganic reinforcement materials (e.g. glass fibre) with abundantly available and less expensive natural fibres. It is important to note, however, that there are sometimes high costs associated with the production of nanofibre fillers from natural materials that can be comparable to the production of some synthetic nanofibres (Johansson *et al*., 2012). Although polymers that are reinforced/filled with natural fibres are generally not as strong as those reinforced with fillers such as cellulose nanofibres, the moderate strength and light weight characteristics of the former still make these suitable to be implemented in food packaging applications, particularly for ready-to-eat food products (Pilla, 2011).

Bast fibres obtained from the outer stem layer of the kenaf plant (*Hibiscus cannabinus* L.) have good mechanical properties that enable this material to be used as a filler and reinforcing agent in biopolymer composites (Abdul Khalil *et al*., 2010; Saba *et al*., 2015). Moreover, the incorporation of natural fibres into PLA matrices in order to reduce material costs and improve mechanical properties has recently gained attention. Furthermore, such composites can be produced *via* commercial plastic processing methods and the incorporation of the natural fibres is believed to enhance compostability and biodegradability (Halász and Csóka, 2012; Kwon *et al*., 2014). In the current study, a novel ternary system comprising PLA, kenaf fibres and thymol, was investigated with a view towards developing a biodegradable and active packaging material.

1.2 Aims of this Work

The overall aim of this study is to develop an AM packaging system from PLA-filled kenaf fibre bio-composites that incorporates a natural AM agent such as thymol. The specific aims are as follows:

- To prepare AM PLA and PLA/kenaf composites *via* melt blending and heat pressing and to determine the mechanical, thermal and morphological properties of these materials at various kenaf loadings and thymol concentrations;
- To evaluate the degradability of AM PLA and PLA/kenaf composites under controlled composting conditions;
- To investigate the interaction of thymol between the PLA matrices and kenaf fibres filler and to determine the retention of thymol in the PLA-based materials following thermal processing;
- To investigate the migration of AM agent from a ternary system comprised of PLA, kenaf fibres and thymol by diffusion, kinetics analyses and Fick's law modelling;
- To examine the temperature dependency of the release rate of thymol from the PLA and PLA/kenaf composite films into fatty and aqueous food simulants;
- To evaluate the effectiveness of AM PLA and PLA/kenaf composites incorporated with thymol against *E. coli* or inoculated on the surface of processed meat samples;
- To investigate the loss of thymol to the atmosphere from these packaging materials and of their thymol retention during storage.

1.3 Thesis Outline

This thesis is comprised of seven papers that are presented in separate chapters. Declarations of co-authorship and co-contribution of these papers are included at the start of the respective chapters. Five of these papers have been published in peer-reviewed journals with a further two manuscripts submitted.

Chapter 2 presents the literature review with an emphasis on PLA-based materials intended for use in AM packaging applications. This chapter reports the recent findings of the engineering characteristics and the AM activity of PLA-based materials incorporated and/or coated with AM agents. The preparation of PLA-based materials incorporated with different AM agents and by using various techniques is also reviewed and critically discussed in this chapter.

In Chapter 3, the effects of untreated and alkali treated kenaf fibres incorporated into the PLA containing thymol in a range of 5 to 10% w/w are examined. The compatibility between the treated kenaf and the PLA matrix as depicted by micrograph images is reported. In this chapter, the PLA/kenaf composites containing untreated and/or treated kenaf has been fixed at 30% w/w kenaf loading. The findings of the mechanical, thermal and morphological properties of the PLA and PLA/kenaf composites containing either no kenaf or no thymol are also discussed. The degradation properties of the studied materials under controlled composting conditions are also presented in this chapter.

It is important to note that the PLA systems containing treated kenaf were selected as the main composite formulation in which to systematically investigate the mechanical and thermal properties, the interaction and retention of AM agent, the migration rate of AM agent from the material and the AM efficacy in laboratory media and on a real foodstuff. Such systems are reported in the remaining chapters (4 to 7).

Chapter 4 provides a detailed examination on the effect of different kenaf fibre loadings ranging from 10 to 40% w/w and thymol concentrations in a range of 5 to 10% w/w on the mechanical, thermal, rheological and morphological properties of the AM materials. The possible packaging applications such as rigid and/or flexible packaging as well as coatings of this particular material can be postulated from these studies.

A standalone Chapter 5 provides an insight into polymer degradation under non-isothermal conditions presented by two novel algorithms. The algorithms are validated using model data and applied to thermogravimetric (TG) data obtained during the degradation of PLA and PLA/kenaf composites containing thymol under non-isothermal conditions. The novel kinetic approach developed in this chapter was used in subsequent chapters to investigate the nature of the interactions between the various components of the composite systems.

Chapter 6 examines the retention of thymol in PLA and PLA/kenaf films following thermal processing by using thermogravimetric and solvent extraction techniques. A detailed discussion of the interactions amongst the PLA matrix, the kenaf fibres and the AM agent in these ternary systems is presented in this chapter. The potential applications of these systems as AM food-packaging materials are also considered.

The diffusivity characteristics of thymol incorporated into the packaging materials with the aim to prolong the shelf life of food products are discussed in Chapter 7. The migration of thymol from the AM PLA and PLA/kenaf films into food simulants is examined. The food simulants used in this study were 15% v/v ethanol/water mixture and 95% v/v ethanol/water mixtures with these simulants representing aqueous and fatty foods respectively. The release rates of thymol into the simulants at different temperatures were determined by using first-order kinetics analysis, diffusion modelling and Fick's law modelling and these are compared and discussed in this chapter.

Chapter 8 examines the effectiveness of AM PLA and PLA/kenaf composite films incorporated with thymol against *E. coli* or inoculated on the surface of processed meat samples. The influences of thymol concentration and kenaf fibres loading on the AM activity of the films are also reported. The capability of PLA and PLA/kenaf films containing thymol as an AM food packaging system is explored in this chapter. Moreover, the loss of thymol to the atmosphere from these packaging materials and their thymol retention during storage under different storage conditions is examined in this chapter.

Finally, the overall conclusions of this work, the significance of the findings as well as the recommendations for future research are stated in Chapter 9.

Chapter 2 – Literature Review

A review of Poly(Lactic Acid)-Based Materials for Antimicrobial Packaging

Overview

Chapter 2 presents the literature review of PLA-based materials intended for use in AM food packaging applications. The engineering characteristics and the AM activity of PLA-based materials incorporated and/or coated with AM agents are reported in this chapter. The current trends in the production of PLA-based materials containing AM and/or antioxidant agents are also presented and discussed.

The paper entitled "A Review of Poly(Lactic Acid)-Based Materials for Antimicrobial Packaging" by Tawakkal I. S. M. A., Cran M. J., Miltz J. and Bigger S. W. was published in the *Journal of Food Science,* **79**(8), 1477-1490, 2014.

PART B:

DECLARATION OF CO-AUTHORSHIP AND CO-CONTRIBUTION: PAPERS INCORPORATED IN THESIS BY PUBLICATION

This declaration is to be completed for each conjointly authored publication and placed at the beginning of the thesis chapter in which the publication appears.

Declaration by [candidate name]: **Signature:** [redacted] **Date:** 15.09.2014

Intan S. M. A. Tawakkal

Paper Title:

A Review of Poly(Lactic Acid)-based Materials for Antimicrobial Packaging

In the case of the above publication, the following authors contributed to the work as follows:

Name	Contribution %	Nature of Contribution
Intan S. M. A. Tawakkal	70	Prepared major part of the manuscript
Marlene J. Cran	10	Contribution to writing of paper and journal submission
Joseph Miltz	10	Contribution to writing of paper
Stephen W. Bigger	10	Contribution to writing of paper

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DECLARATION BY CO-AUTHORS

The undersigned certify that:

1. They meet criteria for authorship in that they have participated in the conception, execution or interpretation of at least that part of the publication in their field of expertise;
2. They take public responsibility for their part of the publication, except for the responsible author who accepts overall responsibility for the publication;
3. There are no other authors of the publication according to these criteria;
4. Potential conflicts of interest have been disclosed to **a)** granting bodies, **b)** the editor or publisher of journals or other publications, and **c)** the head of the responsible academic unit; and
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Tawakkal, I.S.M.A., Cran, M.J., Miltz, J. and Bigger, S.W. (2014), A Review of Poly(Lactic Acid)-Based Materials for Antimicrobial Packaging. Journal of Food Science, 79: R1477-R1490. doi:10.1111/1750-3841.12534

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Chapter 3 – The Influence of Fibre Chemical Treatment

The Influence of Chemically Treated Natural Fibers Containing Thymol in Poly(Lactic Acid)-Based Antimicrobial Composites for Packaging

Overview

Chapter 3 presents a study of the effect of untreated and alkali treated kenaf fibres in PLA/kenaf composites with and without thymol. In this chapter, the preparation of PLA/kenaf composite formulations was fixed at 30% w/w kenaf loading. The findings of the mechanical, thermal and morphological properties of the PLA and PLA/kenaf composites are discussed. The degradation properties of the studied materials under controlled composting conditions are also reported and discussed.

The manuscript entitled "The Influence of Chemically Treated Natural Fibers Containing Thymol in Poly(Lactic Acid)-Based Antimicrobial Composites for Packaging" by Tawakkal I. S. M. A., Cran M. J. and Bigger S. W. was submitted for peer review in the journal, *Polymer Composites*, 2016.

Note:
At the time of submission, this work was under review but has since been published in Polymer Composites, Tawakkal, I. S. M. A., Cran, M. J., & Bigger, S. W. (2016). The Influence of Chemically Treated Natural Fibers in Poly(Lactic Acid) Composites Containing Thymol (early view article) and is attached following this chapter. Moreover, an article for the Society of Plastics Engineers (SPE) online magazine based on this paper/chapter which further demonstrates its impact on the field is included following the full publication.

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The Influence of Chemically Treated Natural Fibers Containing Thymol in Poly(Lactic Acid)-Based Antimicrobial Composites for Packaging

In the case of the above publication, the following authors contributed to the work as follows:

Name	Contribution %	Nature of Contribution
Intan S. M. A. Tawakkal	70	Designed the experiment Performed the sample analyses Prepared major part of the manuscript
Marlene J. Cran	20	Contribution to writing of paper and journal submission
Stephen W. Bigger	10	Contribution to writing of paper

DECLARATION BY CO-AUTHORS

The undersigned certify that:

1. They meet criteria for authorship in that they have participated in the conception, execution or interpretation of at least that part of the publication in their field of expertise;
2. They take public responsibility for their part of the publication, except for the responsible author who accepts overall responsibility for the publication;
3. There are no other authors of the publication according to these criteria;
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The Influence of Chemically Treated Natural Fibers in Poly(Lactic Acid) Composites Containing Thymol

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3.1 Abstract

The mechanical, thermal and morphological properties of poly(lactic acid) (PLA) composites incorporated with 30% w/w untreated kenaf (UK) or alkali treated kenaf (TK) fibers was explored together with the inclusion of 5-10% w/w thymol for potential antimicrobial packaging material applications. The TK fiber composites had significantly higher tensile strength than those containing UK fibers. Scanning electron micrograph images suggested a better adhesion between the TK fibers and the matrix was achieved resulting in improved reinforcement of the PLA/TK fiber composite. The neat PLA and composites containing 10% w/w thymol exhibited lower processing torque, tensile strength and glass transition temperatures than those without thymol suggesting a possible lubricating and/or plasticizing effect. However, the incorporation of thymol into PLA at this level as well as into the composites did not influence the flexibility of the materials as a whole. Decomposition in compost at 58°C progressed rapidly for neat PLA and PLA/thymol resulting in complete disintegration within 35 days. The presence of kenaf slightly inhibited the degradation although complete disintegration of the composite was achieved within 48 days. The results suggest that PLA composites containing kenaf have the potential to be developed as rigid and disposable food packaging materials from biodegradable and renewable resources.

Keywords

Poly(lactic acid); Natural fibers; Polymer composites; Mechanical properties; Thermal properties

3.2 Introduction

To date, many researchers have studied bio-composite materials that are either derived from natural resources or renewable resources for various applications where the focus has been on creating materials that have a perceived low environmental impact. Poly(lactic acid) (PLA) is a relatively new and promising bio-based thermoplastic polyester that can be derived from renewable, bio-derived monomers obtained from a range of plants containing polysaccharides (Jamshidian *et al.*, 2010). For packaging applications, in particular, this polymer provides good strength and optical properties, low toxicity, is readily compostable and is easily formed using equipment that processes conventional, commercial polyolefin plastics (Jamshidian *et al.*, 2010; Obuchi and Ogawa, 2011). Although the potential of PLA for use in packaging applications has been established (Tawakkal *et al.*, 2014b), there are some inherent limitations of this material such as its high production costs, poor water vapour and gas barrier properties, low heat resistance and brittleness, that limit its widespread application in this industry (Auras *et al.*, 2005; Suryanegara *et al.*, 2009).

Naturally sourced fibers or lignocellulosic materials such as wood, kenaf, jute, ramie and flax are commonly used as cost-reducing biofillers and/or reinforcements for PLA and can render the PLA more environmentally friendly in the sense that these can enhance the bio/hydro-degradability of the polymer (Gurunathan *et al.*, 2015; Valdés *et al.*, 2014). Furthermore, PLA generally exhibits a better compatibility and interaction with the natural fibers than petroleum-based polymers during processing (Shanks *et al.*, 2004). Thus, the incorporation of natural fibers into PLA matrices has recently gained attention in order to reduce costs, improve mechanical properties as well as enhance compostability and biodegradability (Halász and Csóka, 2012; Kwon *et al.*, 2014). Bast fibers obtained from the outer stem layer of the kenaf plant (*Hibiscus cannabinus* L.) have good mechanical properties that enable this material to be used as a reinforcement in bio-polymer composites as an alternative to glass fibers (Abdul

Khalil *et al.*, 2010). However, the inherent hydrophilicity of this fiber requires that some chemical modifications need to be made in order to impart good surface adhesion between the polymer and the filler. Moreover, for potential applications related to disposable packaging materials such as food service products, alkali fiber treatment is an important fiber processing step. Such treatments are required to: (i) remove impurities thereby producing a safer packaging materials for food contact and (ii) improve the interaction between the polymer matrix and the fibers (Johar *et al.*, 2012). There are several well-established and recommended pre-treatments for kenaf fibers including graft copolymerization, the use of coupling agents, as well as alkali chemical treatments (Johar *et al.*, 2012). Such modifications can improve the wettability of the fibers with polymer matrices, reduce moisture absorption and ease of processing. Nevertheless, the ability of fibers to reinforce PLA is also influenced by their size, quantity and dispersion as well as processing conditions (Gurunathan *et al.*, 2015; Xia *et al.*, 2015)

In addition to the use of biofillers, the use of some naturally derived additives can also provide additional functional properties of PLA packaging materials (Tawakkal *et al.*, 2016). For example, the incorporation of essential oil extracts such as thymol (2-isopropyl-5-methylphenol) into packaging materials may extend the shelf life of packaged food products by minimizing the growth of microorganisms due to the inherent antimicrobial (AM) properties of this extract. Thymol is a well-established natural AM agent that can reportedly exhibit antioxidant and antimicrobial activity against wide spectrum of microorganisms such as bacteria, fungi, mould and yeast (Del Nobile *et al.*, 2009; Ramos *et al.*, 2014b; Tao *et al.*, 2014). For a continuous AM activity, a migratory release system can be used where volatile additives such as thymol, that often seem a safer alternative to synthetically derived chemical additives, are released from the package into the headspace surrounding the food product (Han, 2003). Moreover, the antimicrobial activity of PLA-based materials can be increased with the inclusion of hydrophilic fibers that promote and facilitate the release of the AM agent (Prapruddivongs and Sombatsompop, 2012; Tawakkal *et al.*, 2016). The combination of the main elements in a composite system comprised of a PLA matrix, kenaf and natural AM substances could potentially improve the key properties and contribute to the sustainability of the system as a whole. However, few studies have reported the development of bio-composites containing both natural fibers and AM additives for use as food packaging materials (Tawakkal *et al.*, 2014a).

The aim of this study was to explore the mechanical, thermal and morphological properties of PLA composites incorporated with untreated or alkali treated kenaf fibers and thymol. Moreover, a visual qualitative analysis of the degradation under thermophilic aerobic conditions was also performed as a preliminary assessment of the compostability of these PLA-based composites.

3.3 Materials and Methods

3.3.1 Materials

Film and bottle grade poly(lactic acid) 7001D Ingeo^{TM}; specific gravity 1.24; weight average molecular weight, $M_w = 1.10 \times 10^5$ g mol^{-1} (Othman *et al.*, 2012); melt flow index (MFI) 6 g/10 min at 210°C and 2.16 kg; melting temperature range 145-160°C was purchased from NatureWorks LLC, USA. Short kenaf fiber bundles (bast) were purchased from Ecofiber Industries, Australia. The aspect ratio (L/D) of the kenaf fibers bundle was approximately 9 with an average length of 920 ± 0.40 µm and an average diameter of 100 ± 0.03 µm. The diameter and length of the fiber bundles were determined by using scanning electron microscopy (SEM) with approximately 90 fiber images that were measured and recorded. Generally, raw kenaf bast fibers consist of *ca.* 63.5% cellulose, 17.6% hemicellulose, 12.7% lignin and 4% extractive (Jonoobi *et al.*, 2009). Thymol (T0501, MW = 150.22 g mol^{-1}), a white crystalline substance with purity of 99.5% was purchased from Sigma-Aldrich Pty. Ltd., Australia. Sodium hydroxide (NaOH) (MW = 40 g mol^{-1}) and acetic acid (CH_3COOH) (MW = 60.05 g mol^{-1}) with purity of 100% were purchased from Merck Chemicals, Australia.

3.3.2 Preparation of Composites

The surface treatment of the kenaf was performed by immersing it in 5% (w/v) NaOH for 2 h at room temperature. Acetic acid was used to adjust the pH during the washing and rinsing of the fibers with distilled water. The treated fibers were removed from the solution and dried overnight in an air circulating oven at 105°C. Prior to mixing with the PLA resin, both the treated and untreated fibers as well as the PLA resin were dried in an oven at 60°C overnight before blending with thymol (Ibrahim *et al.,* 2011). Drying of the fibers before mixing is necessary in order to avoid fibers debonding at the fiber-matrix interface due to the presence of moisture on the fibers surface (Gurunathan *et al*., 2015) and drying also avoids hydrolysis of the polymer. Formulations containing a 30% w/w kenaf loading were prepared with thymol concentrations at zero, 5 or 10% w/w.

The neat PLA and PLA composites containing thymol were compounded using an internal mixer with torque recording capabilities (Haake PolyLab OS, Germany) at 50 rpm and 155°C for 8 min. The PLA resin was introduced in the chamber and mixed for *ca.* 2 min to obtain a constant torque. The kenaf fibers and/or thymol were then added step by step in small amounts during the next 7 to 8 min whilst the mixture was continuously mixed. Films and slabs of 0.3 and 3 mm thickness respectively were prepared using a laboratory press (L0003, IDM Instrument Pty. Ltd., Australia). The samples were preheated at 150°C for 2 min and pressed at the same temperature for 3 min under a force of 50 kN before cooling to 30°C. A hand-held micrometer (Hahn & Kolb, Stuttgart, Germany) was used for measuring film thickness.

3.3.3 Imaging of Fibers and Composites

Scanning electron microscopy (SEM) was performed to observe the morphology of the tensile fracture surface of the composites using a JOEL NeoScope (JCM-5000) scanning electron microscope (SEM) instrument under high vacuum and using an accelerating voltage of 10 kV. Prior to imaging, all samples were coated with a thin layer of gold (up to 6 nm) using a NeoCoater device (MP19020NCTR).

3.3.4 Infrared Analyses

A Shimadzu IR Prestige Fourier transform infrared (FTIR) spectrophotometer with attenuated total reflectance (ATR) technique was used to undertake the infrared spectral analyses. All spectra were recorded in absorbance mode in the range of 550-4000 cm^{-1} with a resolution of 4 cm^{-1} and with 32 scans recorded at every point using Happ-Genzel apodization. A small portion of thymol, and untreated and/or treated fibers were mixed in an agate mortar and pestle with a small amount of paraffin oil. The sample was then applied to a KBr disc and its FTIR spectrum recorded. This technique was required in order to avoid agglomeration of the kenaf fibers and to dilute the high concentration of thymol so the respective peaks could be identified. Ten scans were performed for each acquisition.

For FTIR mapping analysis, the spectra were acquired using a Bruker Hyperion 2000 IR microscope (Bruker Optik Gmbh, Ettlingen, Germany) equipped with a liquid nitrogen cooled detector. The apparatus was connected to the infrared microspectroscopy beamline at the Australian Synchrotron (Clayton, Australia). The microscope was coupled to a Bruker Vertex 70 spectrometer (Bruker Optik Gmbh, Ettlingen, Germany). The line scan and spectral mapping were performed on the surface of PLA film containing 10% w/w thymol and were collected in transmission mode by scanning the film using a computer-controlled microscope stage at an aperture of 10 μm × 10 μm with a 25 × IR objective. The spectral data were collected in the IR range 4000-800 cm^{-1} at a resolution of 4 cm^{-1} and with 64 scans. All spectra were processed using Opus 6.0 software (Bruker Optics, Gmbh, Ettlingen, Germany). A two dimensional (2D) IR map was produced using the software, where integrated areas were calculated for bands attributable to the PLA carbonyl group and thymol ring aromatic functional group.

3.3.5 Tensile Testing

Tensile tests were performed using a Model 4301 Instron Universal Testing Machine with a load cell of 1 kN. Eight tensile specimens were prepared from each sample set *via* heat press moulding using a dog-bone die. The tests were conducted at a cross-head speed of 5 mm min^{-1} on dumbbell-shaped specimens in accordance with the ASTM D638 Type V method until tensile failure was detected and at least six replicate specimens were taken as an average in accordance with the standard method. The average tensile strength, tensile modulus and percentage extension at break were measured and calculated using Instron BlueHill Series IX software. The tensile properties were then normalized against PLA control without filler as well as PLA films containing 5% and 10% w/w thymol to enable a comparison between the studied systems.

3.3.6 Thermal Property Testing

A differential scanning calorimeter (Mettler Toledo DSC1) was used to obtain the thermal properties of the PLA and composites under an inert gas (nitrogen) atmosphere. The samples of *ca.* 8-9 mg each were weighed and sealed in aluminium crucibles. The heating was performed over the range 30 to 300°C at a rate of 10°C min^{-1} and with a nitrogen flow rate of 20 mL min^{-1}. The glass transition temperature (T_g), cold crystallization temperature (T_{cc}), melting temperature (T_m), cold crystallization enthalpy (ΔH_{cc}) and melting enthalpy (ΔH_m) were obtained from the DSC thermograms using the first heating scan. The maximum percentage crystallinity (%X_c) of each sample during the DSC heating was determined by calculating the ratio between the melting enthalpy of the sample and that of 100% crystalline PLA (Du *et al.*, 2014) as shown in Equation (1):

$$\%X_c = \Delta H_m / \Delta H_{m0} \text{ x } 100/w \tag{1}$$

where w is the weight fraction of PLA in the formulations and ΔH_{m0} is the enthalpy of melting for 100% crystalline PLA (93.7 J g^{-1}) (Lee *et al.*, 2009; Liao *et al.*, 2007). The samples were also characterized using a thermogravimetric analyser (TGA) (Mettler Toledo TGA/DSC1) in order to determine their thermal stability. The mass-loss curves of the active composites were recorded when heated from 30 to 500°C at a heating rate of 5ºC min^{-1} and at a flow rate of nitrogen of 0.2 L min^{-1}. The data were obtained *via* the first derivative of the original curve in order to record the onset of thermal degradation.

3.3.7 Disintegration Studies

A study of the decomposition under composting conditions was performed on composite samples cut into pieces (20 × 20 × 3mm). Samples were buried in a commercial compost at 5 cm depth in perforated boxes made of PE and incubated in the oven at 58°C. Aerobic conditions were maintained by mixing the compost periodically and by the addition of water to maintain a moisture content as well as by monitoring the relative humidity of headspace above the compost at *ca.* 60% according to ASTM D5338. Samples were removed from the compost after 7, 14, 21, 28, 35 and 48 days, were immediately washed with distilled water to remove traces of compost and then photographed.

3.4 Results and Discussion

3.4.1 Structural Analysis

To confirm the effect of alkali treatment on the kenaf fibers, surface FTIR spectra of UK and TK fibers were obtained and are shown in Figure 3.1. In the spectrum of the UK fibers, a sharp absorption peak at approximately 1740 cm^{-1} is observed which corresponds to the carbonyl group (>C=O) stretch. The existence of this peak in the UK fibers has been ascribed to the acetyl and ester group in hemicellulose or carboxylic acid group in the ferulic and p-coumeric components of lignin (Johar *et al.*, 2012). This particular peak is not apparent in the case of the TK fibers which indicates the elimination of non-lignocellulose components due to the alkali treatment (Cao *et al.*, 2007; Johar *et al.*, 2012). A similar finding was also reported by Cao *et al.* (2007) who treated kenaf fibers with 5 to 15% (v/w) NaOH solution at 25°C for 2 h.

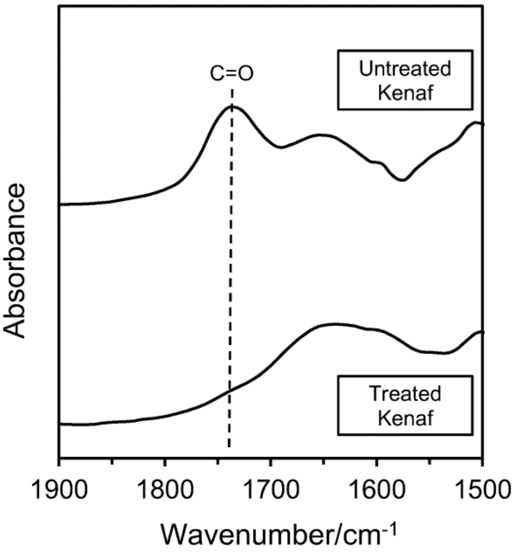

Figure 3.1 Infrared spectra of untreated and treated kenaf fibers in the region 1900-1500 cm^{-1}.

The FTIR structural analysis of thymol and PLA containing 10% w/w thymol in this study have been discussed in detail previously (Tawakkal *et al.*, 2016). However, in that study the presence and distribution of thymol at the surface of the PLA containing 10% w/w thymol film was not investigated and was therefore further analysed using an IR mapping technique in the present study. Figure 3.2 shows the intensity of the IR band mapped over PLA containing 10% w/w thymol film at 1618 and 1700 cm^{-1} that represent the ring aromatic group of thymol and the carbonyl group of PLA respectively (Tawakkal *et al.*, 2016). Figure 3.2(a) shows that a small amount of thymol is present on the film surface with a higher concentration of thymol detected at the left bottom edge of the area tested. This finding is also consistent with the intensity distribution of the PLA carbonyl group (see Figure 3.2(b)) and suggests that this technique can be used to observe the distribution of thymol on the PLA matrix surface and within a relatively small film area. Moreover, the localization of thymol molecules such as within the matrix or the fibers and at the fiber-matrix interface is an important parameter in further understanding the behaviour of thymol based bio-composites but is beyond the scope of the present study.

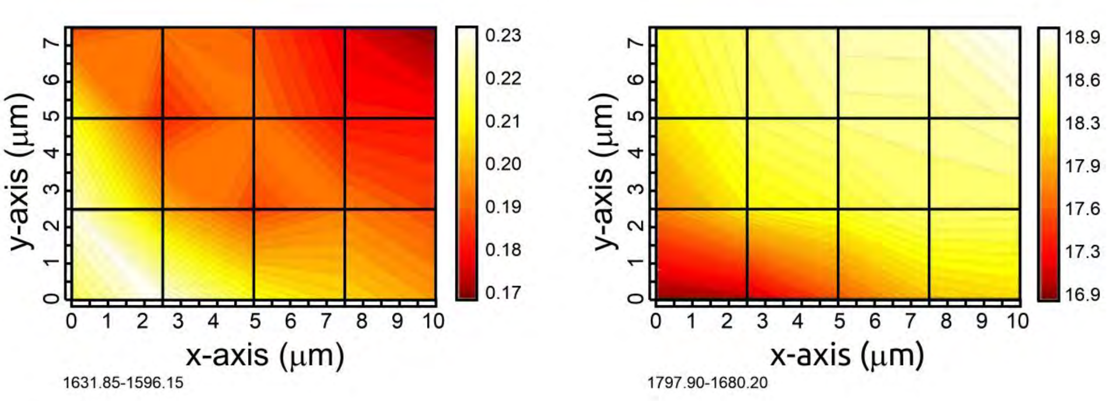

Figure 3.2 Intensity of the IR band mapped over PLA sample containing 10% w/w thymol at: (a) 1622 cm^{-1} (aromatic ring of thymol) and (b) 1700 cm^{-1} (carbonyl group of PLA).

3.4.2 Processing of PLA and Composites

The processing torque profiles of PLA and PLA filled with 30% w/w UK fibers in the presence of thymol are shown in Figure 3.3. Generally, the addition of 5-10% w/w thymol to PLA and PLA/kenaf composites imparts a lubricating effect during processing resulting in a significant reduction in the normalized torque value. This lubricating effect could also be due to the diffusion of thymol molecules to the walls of the internal mixer. Similar trends were observed for active PLA filled with TK fibers containing thymol with no significant changes observed in comparison with the PLA/UK fiber composites (Tawakkal *et al.*, 2014a).

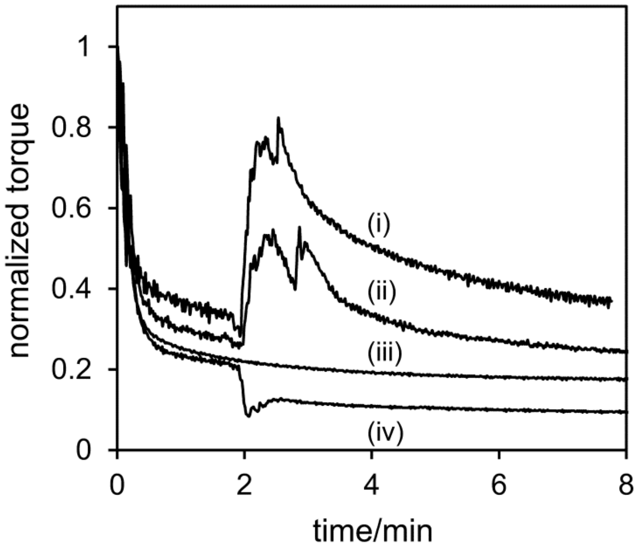

Figure 3.3 Normalized torque as a function of time for PLA melts of: (i) PLA/UK fiber composite, (ii) PLA/UK fiber composite with 10% w/w thymol, (iii) neat PLA and (iv) PLA with 10% w/w thymol.

The peak in the melt torque observed at *ca*. 2 min for PLA composite containing UK fibers (see Figure 3.3) corresponds to the time when the fibers are introduced into the mixer but this peak is absent when thymol is incorporated into the PLA formulation. For the PLA containing thymol, the torque decreased momentarily and achieved an equilibrium torque value almost immediately where it was maintained throughout the mixing process. These results clearly suggest that the thymol acts as a lubricant for the system. Furthermore, this finding is also supported by the significantly lower equilibrium torque observed after *ca*. 8 min in the formulation containing thymol compared with the formulation without thymol. Similar findings have been reported by Sungsanit (2011) who studied the rheology of plasticized PLA blends that contain the oligomer polyethylene glycol (PEG) at 5-20% w/w content. It was found that higher loadings of PEG in the PLA blends produced a lower melt viscosity compared to neat PLA.

It is interesting that the presence of thymol, which is a relatively small molecule compared to typical PEG oligomers, has a noticeable effect on the viscosity of the melt. The lower PLA melt viscosity observed in the presence of 10% w/w thymol may be due to the thymol weakening the intermolecular forces between adjacent polymer chains, spacing them further apart and creating a greater free volume (Sungsanit, 2011). The hydroxyl group in the thymol is also expected to develop a hydrogen bond with the polymer and thus interfere with the polymer-polymer interactions thereby acting in a similar fashion to that of an oligomeric plasticizer (Cao *et al*., 2009).

3.4.3 Composite Morphology

The aim of chemical treatment of the fibers is to remove impurities, pectin, waxy substances, lignin and hemicelluloses in order to create a rough fiber surface and increase hydroxyl group accessibility for a better interaction with the matrix (Mwaikambo and Ansell, 1999). Hydrogen bonding is also likely to occur in the composites between the hydroxyl groups in the UK and TK fibers, the terminal hydroxyl groups of PLA and the carbonyl groups of the ester linkages of PLA (Bax and Müssig, 2008). Figure 3.4(a) and (b) show SEM micrographs of the tensile fracture surfaces of PLA composites containing TK fibers with and without thymol. For both of these composites, it appears that the fibers are well dispersed throughout the polymer matrix. For the PLA composite containing no thymol (Figure 3.4(a)), the TK fibers appear to be more tightly bound to the matrix compared to the composite in which thymol is present (Figure 3.4(b)). This observation is in agreement with the work of Yousif *et al*. (2012) who treated kenaf fibers with 6% (w/v) NaOH and observed a slight improvement in the interfacial adhesion and the porosity of the epoxy/kenaf composites which prevented the debonding, detachments or pull-out of fibers. The presence of thymol in the matrix appears to facilitate the slippage of the fibers out of the matrix during fracture and this is seen in Figure 3.4(b) with the presence of more protruding fibers at the matrix surface. Moreover, the thymol that is present on the surface of the kenaf fibers will lower the coefficient of friction and further facilitate the slippage of the fibers within the matrix. The smooth surfaces of some of the fibers, as well as the voids, suggests that there is weaker adhesion between the fibers and the PLA matrix when thymol is present in the formulation compared to systems where thymol is absent.

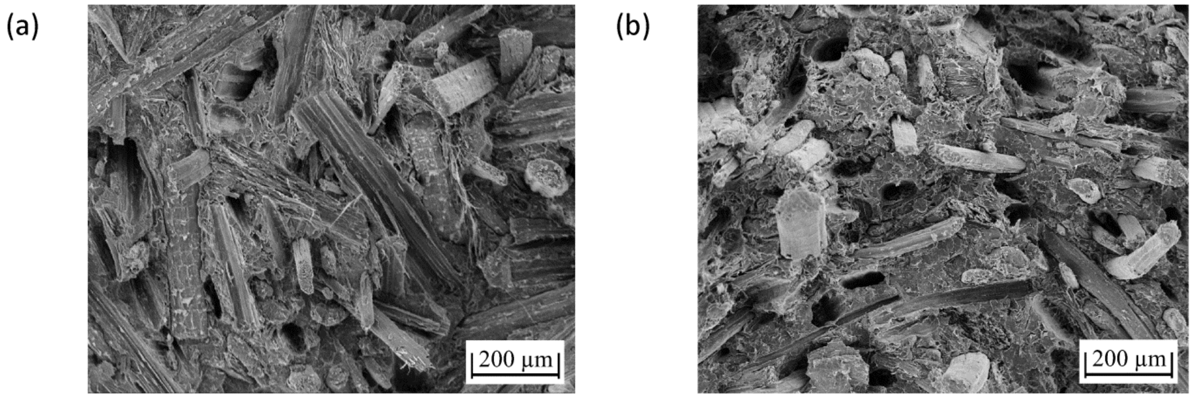

Figure 3.4 Scanning electron micrographs of PLA composites of: (a) PLA/TK fiber composite and (b) PLA/TK fiber composite with 10% w/w thymol at 200× magnification.

3.4.4 Mechanical Properties

Figure 3.5 shows the effect of the presence of UK or TK fibers on the normalized tensile properties of PLA composites containing zero, 5 or 10% w/w thymol. The tensile strength of PLA filled with TK fibers and containing no thymol was slightly higher than that of the UK composites as well as that of the neat PLA (see Figure 3.5(a)). The TK fibers act as reinforcement and impart an approximately 8% increment in tensile strength, whereas no significant changes in the tensile strength of the neat PLA were imparted in the case of the composite containing the UK fibers. The slight improvement in the tensile strength is possibly due to better interfacial adhesion between the matrix and the TK fibers as well as better mixing or compatibility within the composite system (Ibrahim *et al.*, 2011; Xia *et al.*, 2015).

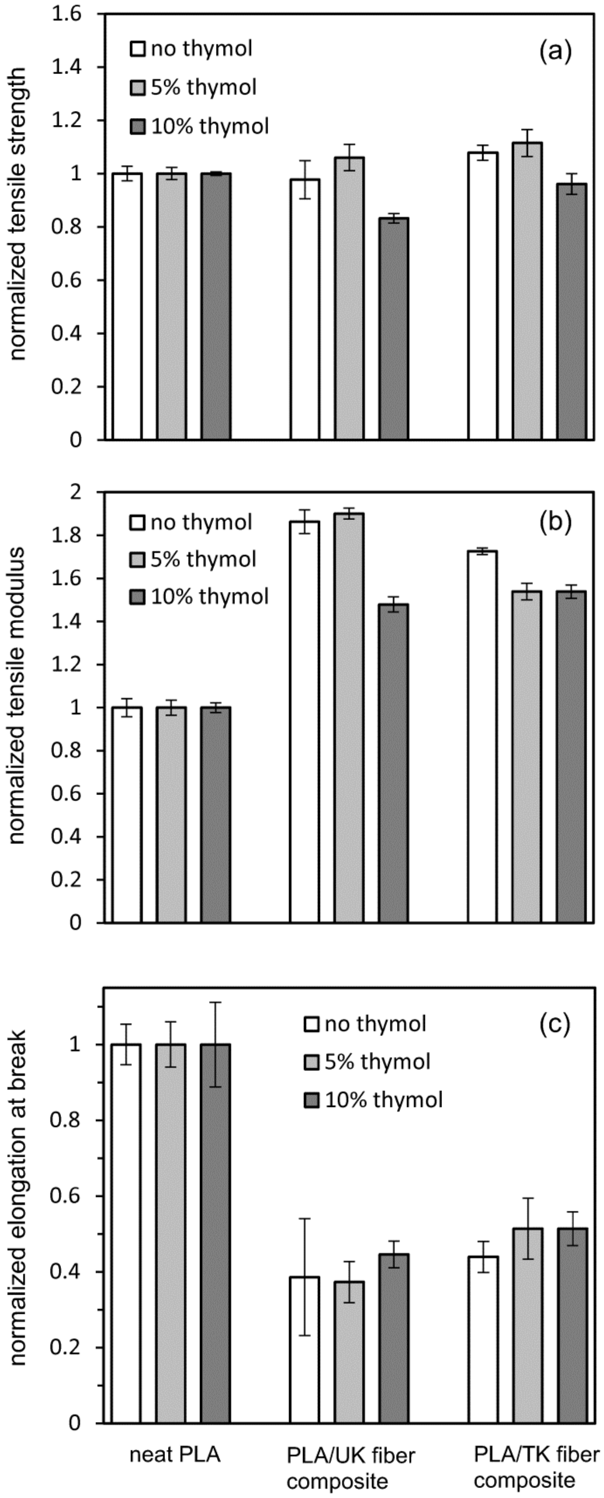

Figure 3.5 Normalized tensile properties of PLA composites with UK or TK fibers containing zero, 5 or 10% w/w thymol: (a) tensile strength; (b) tensile modulus; and (c) percentage elongation at break.

The addition of 10% w/w thymol into the neat PLA and the PLA/kenaf composites containing UK and TK caused a significant reduction in the tensile strength compared to those composites without thymol. The percentage reduction in the tensile strength was found to be 11%, 24% and 21% for neat PLA, and PLA composites containing UK and TK fibers, respectively. The localized plasticizing effect between the PLA and the thymol whereby the thymol molecules diffuse into the bulk of the matrix between the PLA chains is in some respects akin to the case of PLA containing low molecular weight PEG plasticizer (Chieng *et al.*, 2013). This suggests that the thymol additive interferes with the interaction between the polymer matrix and the fiber in the presence of the applied stress due to the aforementioned slippage effect (Ramos *et al.*, 2012). This finding is consistent with the observations made in assessing the SEM micrograph images in Figure 3.4(b). In addition, the latter composites demonstrated lower tensile strength than the neat PLA containing only 10% w/w thymol. Thus, thymol may increase end slipping of fibers from the polymer matrix and subsequently reduce the tensile strength. Similarly, Taib *et al.* (2009) reported a reduction up to 15% in the tensile strength of PLA/30% w/w kenaf composites containing 10% w/w PEG. Interestingly, the PLA/kenaf composite containing TK fibers and 10% w/w thymol exhibited a higher tensile strength (51 MPa) than the composite containing UK fibers and 10% w/w thymol (44 MPa). This suggests that the reinforcement offered by the TK fibers may prevail over the fiber slippage effect of thymol in PLA at this level of additive in the system. In addition, changes in the tensile strength that may arise due to the effect of the thymol may not be significant at a low level of thymol (5% w/w).

The effect of UK and TK fibers on the normalized tensile modulus and elongation at break of the composites containing zero, 5 or 10% w/w thymol is shown in Figure 3.5(b) and (c) respectively. As expected, the tensile modulus (or "stiffness") of the composites containing UK or TK fibers is significantly greater than that of the neat PLA by *ca*. 80% (see Figure 3.5(b)).

This may be due to the inherently high stiffness of the fiber (Suryanegara *et al*., 2009), and this finding is also in agreement with the work of Sujaritjun *et al*. (2013) who incorporated PLA with 30% w/w bamboo fibers and found an increase in the tensile modulus of *ca*. 17%. In this case, the tensile modulus of PLA filled with TK fibers was slightly lower than the composite containing UK fibers. A similar finding was observed by Xia *et al*. (2015) who investigated the tensile modulus of PLA composites containing untreated and alkaline treated jute fibers reinforcements. Cao *et al*. (2007) reported that kenaf fibers treated with 5% NaOH solution are of smaller fiber diameter, increasing the tensile strength of the fiber with no significant changes in the tensile modulus when compared with untreated kenaf fiber. Nevertheless, it is important to note that the removal of surface components from the fibers during the alkaline treatment cannot be solely responsible for the observed decrease in the stiffness of the composite systems. This is because the stiffness of composites containing natural fibers is mainly related to the cellulose microfibrils which are the major structural components within the microstructure. The reduction in tensile modulus also occurs upon the addition of 10% w/w thymol to the systems containing kenaf, with no changes in the tensile modulus observed upon the addition of 10% w/w thymol to neat PLA. This finding is in sharp contrast to the findings of Ramos *et al*. (2014b), who reported a 15% reduction in the tensile modulus of PLA films impregnated with 8% w/w thymol, although the reasons for the difference in findings are unclear. Nonetheless, the decrease in the stiffness of the composites containing UK and TK fibers are more pronounced due to the effect of the thymol as evidenced in Figure 3.4(d).

Figure 3.5(c) shows the effect of UK and TK fibers on the normalized elongation at break of composites containing zero, 5 or 10% w/w thymol. In general, the elongation at break of these composites decreases with the addition of the fibrous filler. This suggests that the kenaf does not contribute to the elasticity or the final composite flexibility and this observation is likely to be related to the high stiffness of the composites as indicated by the results in Figure 3.5(b). In contrast, the elongations at break of PLA/flax composites were found to be 100% higher than neat PLA, with no significant changes having been found between the untreated and treated PLA/flax fibers composites (Xia *et al*., 2015). No significant changes in the elongation at break of any of the composites containing UK and TK fiber were observed upon the addition of 5-10% w/w thymol. Liu *et al*. (2010) incorporated 5% w/w loadings of both Nisaplin and EDTA into plasticized PLA/glycerol triacetate films and observed a significant reduction of the elongation at break from 108.5 to 62.5% compared to the plasticized PLA. However, a slight increase in the elongation at break was found in extruded PP containing 8% w/w thymol resulting in an increase in ductile properties (Ramos *et al*., 2012). The reason for this difference in behaviour of the two systems is unclear, nonetheless it is possible that a higher loading of 10% w/w thymol in PLA may produce an enhancement of the elasticity properties of the system. This has not been verified in the present study.

In the case of PLA/kenaf composites containing thymol, it appears that the presence of thymol does not contribute to the flexibility of the composites as a whole. This finding is supported by the work of Taib *et al*. (2009) who prepared PLA plasticized with PEG and found a higher strain at break (42%) compared to neat PLA (4%), whereas the addition of 30% w/w kenaf in the plasticised PLA reduced the stain at break significantly to 1%. The addition of UK and TK significantly reduces the flexibility and has been attributed to the stiffening effect of kenaf. The addition of kenaf to the system restricts the mobility of PLA chains and increases the number of the stress-concentrated areas at the fiber ends that can ultimately contribute to the mechanical failure of the composite (Taib *et al*., 2009). The further inclusion of a small molecular species such as thymol essential oil to the PLA/kenaf composite system undoubtedly increases the complexity of the system and further complicates any explanation of the experimental observations. Nonetheless, it can be suggested that the type of active PLA/kenaf composite under investigation in the present study is more suitable for use as a rigid packaging material than a flexible packaging material due to the high stiffness of this material (Faludi *et al.*, 2013).

It is important to note that the mechanical properties of these composite systems also depend on the fiber size distribution and/or aspect ratio of the fibers. High shear stresses developed during the compounding with an extruder or mixer may lead to fiber damage or breakage resulting in a smaller fiber aspect ratio (Kwon *et al.*, 2014). Moreover, alkaline treatments reduce the fiber diameter and thereby increase the fiber aspect ratio (Cao *et al.*, 2012). The effect of fiber size distribution of the alkali treated fibers containing AM additive composites on the mechanical properties should be considered in the future in order to fully understand the complexities of stress transfer in these ternary composite systems.

3.4.5 Thermal Properties

Table 3.1 summarises the key data obtained from the analysis of the DSC thermograms of PLA and PLA composites containing UK or TK fibers with zero, 5 or 10% w/w thymol. Neat PLA exhibited an onset T_g at 59.5°C, a T_{cc} at 93.3°C and T_m values at 142.3 (shoulder) and 149.6°C (peak), the latter of which is higher than the value of 141.7°C reported by Byun *et al.* (2010). The onset of T_g, T_{cc}, T_m of the PLA composites containing UK or TK fibers demonstrated no significant differences. However, some of these values, such as T_g, T_{cc}, T_m, ΔH_{cc} and ΔH_m, for both of the composites were slightly lower than that of neat PLA. The maximum values of $\%X_c$ calculated using equation (1) for the composites containing UK or TK fibers was slightly higher than that of neat PLA and this finding is in agreement with the work of Du *et al.* (2014) who investigated PLA containing 30% w/w pulp fibers from hardwood, softwood and bleached Kraft wood.

Table 3.1 Thermal analysis parameters obtained from DSC thermograms of neat PLA, PLA/kenaf and PLA/kenaf/thymol composites.

Formulation %w/w thymol	T_g /°C	T_{cc} /°C	T_{m1}/°C	T_{m2}/°C	ΔH_{cc}/J g^{-1}	ΔH_m/J g^{-1}	X_c /%
Neat PLA							
0	59.5	93.3	142.3[a]	149.6	18.8	26.1	28.0
5	47.9	86.9	131.9	143.0	20.0	23.4	26.7
10	41.6	85.6	124.5	137.4	22.1	22.7	27.1
PLA/UK fibers							
0	59.6	93.5	138.1	146.2	19.7	19.4	29.8
5	46.6	82.9	123.3	134.6	16.0	15.9	26.3
10	30.1	75.7	115.5	129.3	15.9	15.7	28.1
PLA/TK fibers							
0	58.8	94.9	137.8	145.5	19.0	19.1	29.2
5	46.9	85.9	125.5	136.18	16.3	16.6	27.4
10	31.3	78.5	116.7	129.8	15.8	15.8	28.3

[a] Shoulder on the main PLA peak

Figure 3.6 presents the DCS thermograms of the PLA and PLA composite systems and, as shown in Figure 3.6(a), the neat PLA exhibits a double melting peak with a dominant peak at higher temperature (T_{m2}). With the addition of kenaf fibers, the double melting peaks become more distinct. The appearance of these dual melting peaks is in accordance with other reports where the addition of fibers resulted in the development of a small melting peak that shifted to a lower temperature, T_{m1}, upon further addition of fibers (Du *et al.*, 2014; Prapruddivongs and Sombatsompop, 2012; Tawakkal *et al.*, 2014a). The minor peak may be due to either a different PLA crystal type formed only in presence of the fibers and/or melting of the transcrystalline zone as reported by Yussuf *et al.* (2010). For the latter, this may also be due to recrystallization of PLA crystals of different lamella thicknesses that can be verified by using DSC at different heating ramp rates. Moreover, there are several other factors that can lead to multiple melting peaks and these may have arisen from the melting, recrystallization, and remelting processes during heating, the presence of more than one crystal type, different polymer morphologies, different molecular weight species, the effects of orientation as well as physical aging.

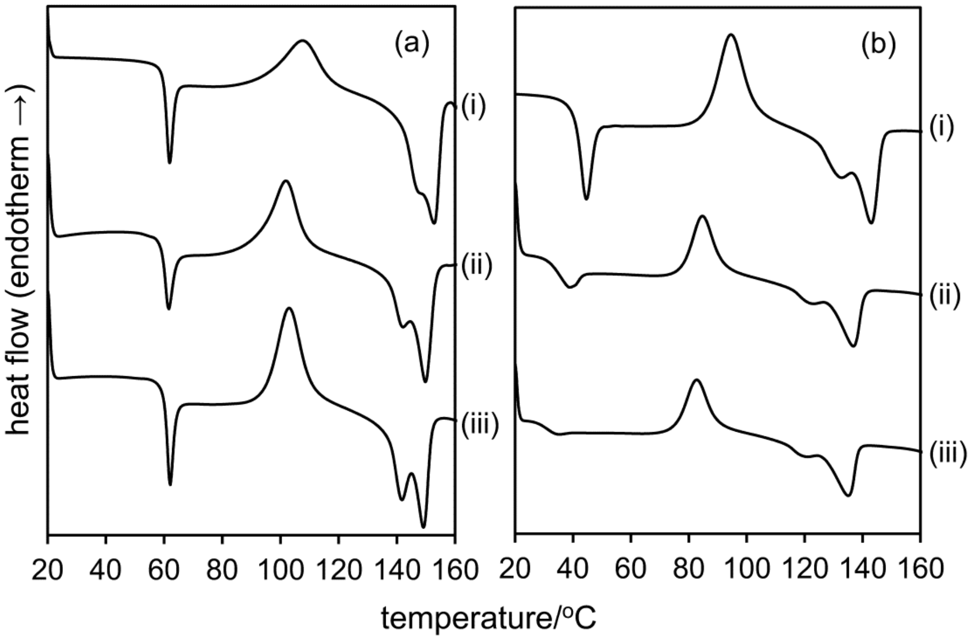

Figure 3.6 DSC thermograms of PLA and PLA composites (a) neat PLA and PLA composites without thymol: (i) neat PLA, (ii) PLA/UK fiber, (iii) PLA/TK fiber and (b) neat PLA and PLA composites with 10% w/w thymol: (i) neat PLA, (ii) PLA/UK fiber, (iii) PLA/TK fiber.

For the PLA and PLA composites containing 5-10% w/w thymol, the addition of the additive resulted in a decrease in T_g (see Table 3.1 and Figure 3.6(b)). This might be attributed to the resultant increase in the free volume of polymer matrix as well as segmental mobility of the PLA chains that change the thermal properties in a way that is akin to a plasticizing effect (Jamshidian *et al.*, 2012; Tawakkal *et al.*, 2014a). The incorporation of thymol in the PLA decreases the T_g value which may be a similar effect as that previously observed in the case of PLA containing low and high molecular weight PEG at a 10% w/w loading (Chieng *et al.*, 2013; Taib *et al.*, 2009). Furthermore, Halász and Csóka (2012) reported a similar finding where the addition of 10% w/w PEG decreased the T_g of neat PLA and PLA composite containing 5% w/w microcrystalline cellulose (MCC). The observed plasticizing effect reflected in the T_g values indicates that the thymol is solubilised with the PLA and this is consistent with the observations made from the morphology images, tensile strength and tensile modulus of neat PLA and PLA kenaf composites (UK and TK) containing thymol (see Figure 3.4(b) and Figure 3.5). It can be observed that, as the content of thymol was increased to 10% w/w in the PLA film, the T_{cc} peak shifted to slightly lower temperature, was slightly sharpened, and the ΔH_{cc} value was increased (see Figure 3.6 and Table 3.1). This may be attributed to a nucleating effect where the presence of the additive enhances the ability of PLA to undergo cold crystallization as reported for more complex composite PLA films (Silverajah *et al.*, 2012).

Two melting peaks were also observed for PLA containing thymol. The additional melting peak exhibited by the active PLA formulation is more pronounced than that of the neat PLA (see Figure 3.6) and this may also be related to the reorganization of the crystal structure. These observations contrast with other findings in the literature where no additional melting peak arose upon the addition of plasticizer such as low molecular weight PEG200 in PLA blends (Chieng *et al.*, 2013). In general, slight decreases were observed for the T_g, T_{cc}, and T_m of PLA/kenaf composites containing 10% w/w thymol irrespective of the fiber pre-treatment.

3.4.6 Thermogravimetric Analysis

It is important to investigate the decomposition and degradation of composites at higher temperature, especially if these materials are intended for use in food packaging applications. Low levels of degradation products produced during the thermal processing of such polymers may taint and/or contaminate foodstuffs upon contact thereby causing concerns regarding organoleptic properties or indeed safety. Furthermore, the thermal stability of these materials is also of relevance to the ultimate disposal in composting or land fill where degradation usually takes place at elevated temperatures.

The thermogravimetric (TG) profiles of neat PLA and PLA composites containing UK and TK fibers with zero or 10% w/w thymol are shown in Figure 3.7 in the form of the normalized weight loss as a function of temperature. The corresponding derivative weight loss curves are also shown in Figure 3.7 to enable a more detailed analysis of the TG data to be made. The thermal degradation of neat PLA takes place in a single step with a maximum rate of weight loss in the range of 280 to 355°C (Ramos *et al*., 2014b). In general, the maximum degradation temperature of the neat PLA was higher than any of the PLA composites (see Figure 3.7(a)) and this is more clearly reflected in the derivative TG analysis curves. As expected, the presence of fibers in the PLA destabilised the PLA matrix in the composite as has been previously reported by Yussuf *et al*. (2010) whereby some portion of the polymer is replaced with less thermally stable fibers in the composite materials. The temperature at which the maximum rate of degradation occurs for the composite containing TK fibers was slightly higher than that of the composite containing UK fibers with degradation temperatures of 331 and 328 °C respectively. This may be due to the better interfacial adhesion between the TK fibers and the PLA matrix (Yu *et al*., 2010).

The TG profile of PLA containing a nominal 10% w/w thymol in the formulation exhibits two main steps during the analysis (see Figure 3.7(b)). The first step occurs gradually over the temperature range of *ca*. 30 to 300°C and is attributable to the release of thymol from the polymer matrix. This step corresponds to a *ca*. 7 % w/w loss in total mass and suggests that the neat PLA and PLA composites that were formulated with 10% w/w thymol retain *ca*. 7% w/w of the thymol that was originally added to the formulation. Thymol release during melt processing due to the high friction between the barrel and the screw, is primarily responsible for the loss of thymol from the formulation (Graciano-Verdugo *et al*., 2010). This observation is in agreement with the work by Ramos *et al*. (2014b) who investigated by TG analysis the retention of thymol in a PLA matrix after processing. The temperature corresponding to the maximum rate of release of thymol was slightly higher for the composites containing TK fibers (148°C) compared to those containing UK fibers (141°C) suggesting that the thymol interacts more strongly with the TK fibers. The second step observed in the TG profiles of the PLA and PLA composites containing thymol occurs over the temperature range of *ca*. 310 to 350°C and is attributable to the complete degradation of the polymer matrix.

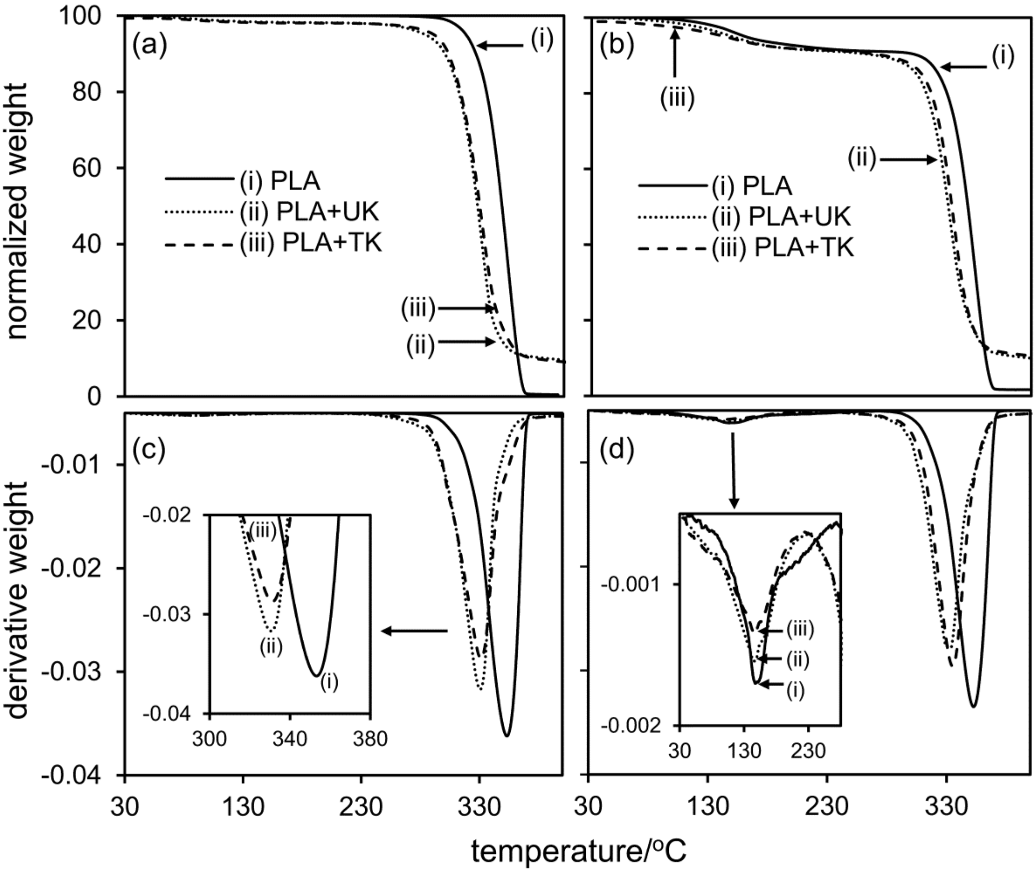

Figure 3.7 TGA profiles of PLA and PLA composites
(a) neat PLA and PLA composites without thymol: (i) neat PLA, (ii) PLA/UK fiber, (iii) PLA/TK fiber and (b) neat PLA and PLA composites with 10% w/w thymol: (i) neat PLA, (ii) PLA/UK fiber, (iii) PLA/TK fiber.

The temperature corresponding to the maximum rate of degradation for the composite containing TK fibers after the release of 10% w/w thymol was slightly higher than that of the composite containing UK fibers. This may be due to the increased thermal stability of the almost complete cellulosic material that comprises the TK fibers and the absence of other components such as waxy substances, hemicellulose and lignin that may otherwise destabilize the system (Ibrahim *et al*., 2011; Shukor *et al*., 2014). Overall, the addition of thymol to the formulation seems to have little effect on the thermal stability of the PLA whereas the addition of fiber decreases the thermal stability.

3.4.7 Decomposition in Compost

Although many studies have focused on the biodegradation and/or composting of PLA and PLA incorporated bio-filler composites, few have investigated the composting of PLA-based materials containing AM agents. Moreover, there is still a question of whether the AM property of the material would compromise or affect the ultimate degradation by the microorganisms in soil (Wang *et al*., 2015). In the present study, a preliminary and qualitative analysis of the disintegration under controlled compost-like conditions of PLA and PLA/kenaf films containing the AM agent thymol was conducted as a prelude to future controlled composting studies of these materials. Figure 3.8 shows images of PLA and PLA composites containing 30% w/w TK fibers with and without 10% w/w thymol that were removed from controlled composting conditions at different times. Overall, a considerable modification in colour of all samples was observed with a change from clear to opaque for the neat PLA and PLA containing 10% w/w thymol and changes were also observed for the composites. A similar observation was reported by Ramos *et al*. (2014a) who investigated the disintegration of PLA and PLA nano-composites containing thymol and silver nanoparticles under controlled composting conditions. These colour changes are primarily due to the hydrolytic degradation and crystallization of the PLA matrix (Pantani and Sorrentino, 2013). Moisture absorption may lead to hydrolysis that breaks down the macro-molecular chains of the polymer with a consequent erosion of the sample surface as demonstrated by the whitening of the surfaces followed by microbial assimilation (Martucci and Ruseckaite, 2015). In addition, the hydrolysis of PLA may also be attributed to the presence of enzymes or catalysts (e.g. carboxylic acid (RCOOH)) as well as the higher temperature conditions in order to promote the reaction and degradation processes. It is important to note that the degradation of PLA can be very slow in soil and this may be due to the limited presence of naturally occurring enzymes required to degrade PLA in the natural environment and at lower temperatures.

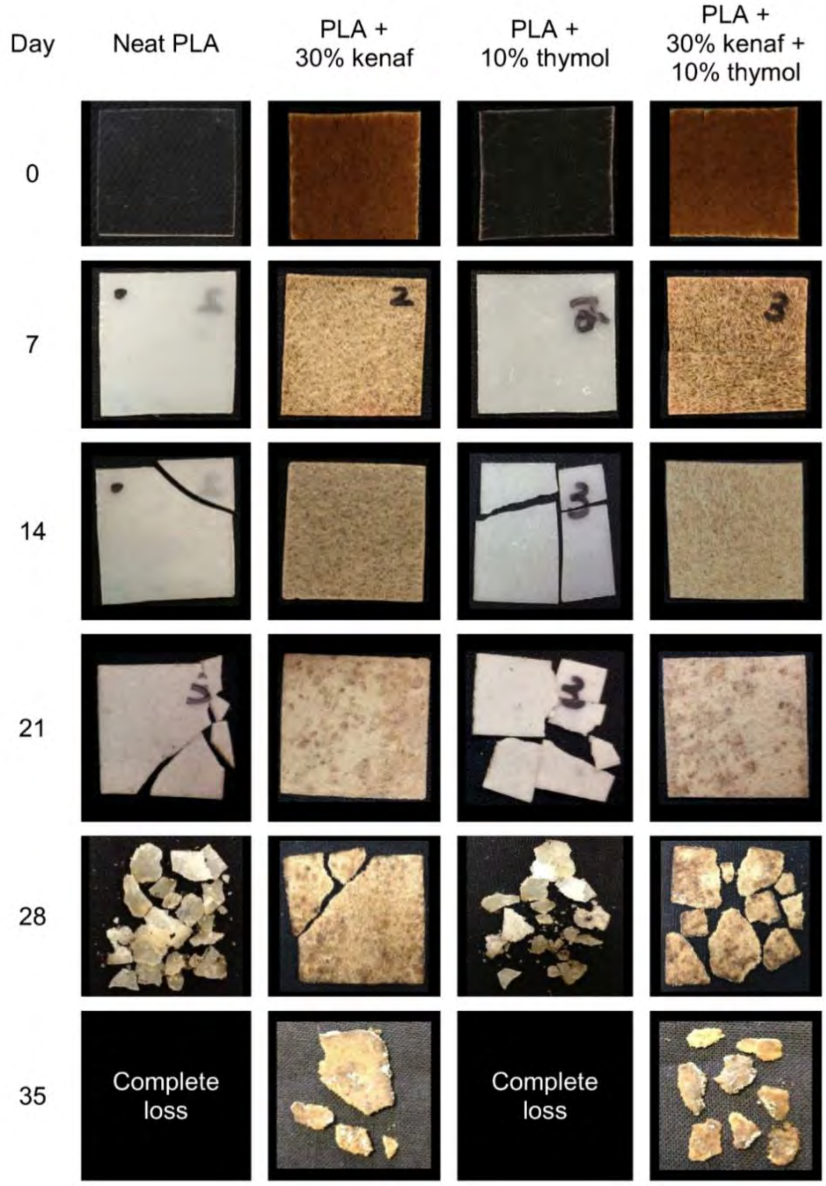

Figure 3.8 Images of PLA and PLA containing TK fiber and/or thymol at different stages of decomposition in compost.

One may expect that the incorporation of AM agent into the PLA-based films will delay the degradation process. However, the neat PLA and PLA containing 10% w/w thymol commenced degrading within 14 days as evidenced by sample fragmentation although no significant differences were observed between the PLA formulations with and without thymol. For composite samples containing kenaf, degradation was not apparent until 28 days under the test conditions and in these cases, the PLA surrounding the fiber was observed to have subsequently degraded, dislodging the fibers and roughening the surface. The differences in the degradation rate of these two systems may be attributed to the diffusion through the composites with the PLA readily adsorbing water and/or the resistance in water uptake with the presence of filler, whereby a constant molecular mobility of water in the permeable (amorphous) phase, forcing diffusion to follow a more tortuous path was created (Azwa *et al.*, 2013; Mathew *et al.*, 2005). The relatively high degradation rate of the PLA under the aerobic composting conditions is not unexpected under the high humidity and temperature (58°C) conditions applied (Mathew *et al.*, 2005). Compost monitoring such as pH, evolved CO_2, and the molar mass of PLA as well as microstructural analysis of the composites by SEM imaging and DSC during biodegradation would be required for further investigation in order to depict the effect of thymol on the biodegradation processes. Moreover, the changes in the properties of the materials could be employed since the overall degradation of PLA is *via* a surface or bulk erosion process.

3.5 Conclusions

Bio-based PLA composites containing TK or UK kenaf fibers and thymol were prepared and characterized. The incorporation of thymol into the PLA/kenaf composites imparted a lubricating effect whereby the equilibrium torque value was decreased during processing. A relatively weak adhesion between the PLA matrix and the kenaf fibers was confirmed microscopically in composites containing thymol. The tensile strength of PLA composites containing TK fibers was slightly higher than that of composites containing UK fibers, suggesting the alkaline treatment imparts a reinforcing effect within the polymer matrix. The incorporation of the higher level of 10% w/w thymol into the PLA/kenaf composites decreased the tensile strength and stiffness irrespective of the fiber pre-treatment with no significant changes on the elasticity. Thermal analysis by DSC showed a general decrease in T_g, T_{cc} and T_m in PLA and PLA composites containing 10% w/w thymol compared to the formulations without thymol suggesting changes in the phase structure of the polymer. The TG decomposition temperature of the PLA composite containing TK fibers and 10% w/w thymol was slightly increased indicating an increase in the thermal stability of the PLA matrix. Under controlled composting conditions, the degradation of the PLA and PLA containing TK fibers progressed rapidly resulting in a complete loss within 48 days. In general, the presence of TK fibers in combination with thymol in the PLA composite resulted in significant improvement in the overall properties of the material suggesting that this composite has the potential to be used as an active bio-based packaging material.

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Chapter 4 – Effect of Kenaf Fibre Loadings

Effect of Kenaf Fibre Loading and Thymol Concentration on the Mechanical and Thermal Properties of PLA/Kenaf/Thymol Composites

Overview

In this chapter, the PLA systems containing treated kenaf were selected as the main formulation with which to further investigate the effect of kenaf fibre loadings and thymol concentrations on the mechanical, thermal and rheological properties of the AM materials. The possible packaging applications such as rigid and/or flexible packaging as well as coatings of this particular material can be suggested from these studies.

The paper entitled "Effect of Kenaf Fibre Loading and Thymol Concentration on the Mechanical and Thermal Properties of PLA/Kenaf/Thymol Composites" by Tawakkal I. S. M. A., Cran M. J. and Bigger S. W. was published in *Industrial Crops and Products*, **61**, 74-83.

PART B:

DECLARATION OF CO-AUTHORSHIP AND CO-CONTRIBUTION: PAPERS INCORPORATED IN THESIS BY PUBLICATION

This declaration is to be completed for each conjointly authored publication and placed at the beginning of the thesis chapter in which the publication appears.

Declaration by [candidate name]: Intan S. M. A. Tawakkal

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In the case of the above publication, the following authors contributed to the work as follows:

Name	Contribution %	Nature of Contribution
Intan S. M. A. Tawakkal	70	Designed the experiment Performed the sample analyses Prepared major part of the manuscript
Marlene J. Cran	20	Contribution to writing of paper and journal submission
Stephen W. Bigger	10	Contribution to writing of paper

DECLARATION BY CO-AUTHORS

The undersigned certify that:

1. They meet criteria for authorship in that they have participated in the conception, execution or interpretation of at least that part of the publication in their field of expertise;
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Effect of kenaf fibre loading and thymol concentration on the mechanical and thermal properties of PLA/kenaf/thymol composites

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ABSTRACT

Composites of poly(lactic acid) (PLA) containing up to 40% (w/w) of kenaf fibre and up to 10% (w/w) of thymol were studied to evaluate the mechanical and thermal properties. These composites were compared with control systems containing either no fibre or no thymol and were prepared using melt blending and compression moulding techniques. The composites with 10% (w/w) thymol had the lowest tensile strength with slightly higher flexibility compared with those systems containing lower concentrations of this additive. The tensile properties of composites containing 5% (w/w) thymol demonstrated that the addition of fibre to the PLA/kenaf composites was affected more than the incorporation of the thymol alone. Thermogravimetric analysis of neat PLA and PLA/kenaf composites incorporated with 5% and 10% (w/w) thymol revealed no significant changes in the decomposition temperature. Analysis by differential scanning calorimetry, however, showed a decrease in all of the key thermal transitions with the addition of 5% and 10% (w/w) thymol into the neat PLA and PLA/kenaf composites. The results of the mechanical and thermal properties tests suggest that thymol acts as a plasticizing agent in this composite.

1. Introduction

The development of active packaging (AP) materials based on biopolymers is expected to grow in the next decade with major focuses on extending the shelf life of foods, enhancing food safety and quality, providing viable alternatives to petroleum based polymers and reducing environmental impacts (Bonilla et al., 2013; Jin and Zhang, 2008). To date, many researchers have studied packaging materials that are either derived from natural resources or are made from renewable resources in order to fulfil the requirements set by some regulatory agencies and/or those of consumers who prefer more environmentally friendly packaging that minimizes pollution (Kuorwel et al., 2011a).

Biopolymers such as poly(lactic acid) (PLA) can be synthesized from renewable, bioderived monomers obtained from a range of materials including starch from corn (Auras et al., 2005). Since PLA is a relatively new biopolymer, considerable effort has been placed into making it as acceptable and effective as its commercial petroleum-based counterparts (Auras et al., 2005). The potential of PLA for use in AP packaging applications has been investigated by a number of researchers (Ali and Noori, 2014; González and Igarzabal, 2013; Jin, 2010; Mustapha et al., 2002; Rhim et al., 2009). Current AP technologies enable effective antimicrobial (AM) packaging materials to be prepared from PLA that have been incorporated or blended with different compatible materials, additives and/or plasticizers.

Several classes of AM additives are used in packaging with those derived from natural sources such as herbs and plant species of particular importance. Thyme, for example, contains volatile essential oils and extracts that are rich in terpenoids, particularly monoterpenoid phenols such as thymol and carvacrol. Thymol (2-isopropyl-5-methylphenol) is known to exhibit antimicrobial activity against several microorganisms such as bacteria, fungi, mould and yeast (Gutierrez et al., 2009) and it is classified in the "Generally Recognized as Safe (GRAS)" category by the US Food and Drug Administration (Persico et al., 2009). Thymol is ideally suited for food packaging applications requiring a migratory release that can provide continuous AM activity from the package to the food (González and Igarzabal, 2013; Kuorwel et al., 2011b; Ramos et al., 2012). Although there are few reports of PLA films and coatings containing AM agents, there are some commercial products available in the marketplace such as the controlled release antifungal PLA/starch film ANTIPACK^{TM} AF (Szafranska, 2013). Of the reported studies, some have investigated the microbial activity of such active films by the diffusion of AM agents in solid and liquid media as well as in related real food products with little attention having been

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devoted to studying the physico-mechanical, thermal and other properties of the active material systems (Bastarrachea et al., 2011).

The combination of natural fibres into PLA is receiving attention mainly for its potential to create materials that are more environmentally sustainable. The use of these natural fibres as fillers or reinforcements in PLA composites is expected to considerably lower the overall cost of the material due to the renewability and abundance of these raw materials (Abdul Khalil et al., 2012a; Akil et al., 2011; Faruk et al., 2012). Furthermore, the production of biopolymers incorporated with natural fibres offers several other advantages including improved compatibility and better mechanical properties, improved abrasion resistance as well as enhanced biodegradability (Anuar et al., 2012; Du et al., 2014; Tawakkal et al., 2012). Several studies have reported the treatment of fibres as a means to create better compatibility between the polymer matrix and the fibres although this has the potential to produce waste chemicals. Treatments include the use of alkaline agents (Huda et al., 2008; Ibrahim et al., 2011) and silane agents (Le Moigne et al., 2014; Tran et al., 2014) which would be expected to alter the surface chemistry of the fibres and promote fibre/PLA compatibility. However, the environmental impact of the chemical treatment of kenaf fibres has not been addressed but it could potentially be overcome by treating the chemical waste before it is released to the environment.

Examples of relatively inexpensive and widely available plant fibres that can be used as fillers include wood flour, jute, hemp, flax, kenaf, bamboo, oil palm empty fruit bunch, rice husk and ramie (Akil et al., 2011; Harmaen et al., 2013; Van Den Oever et al., 2010). Non-wood lignocellulosic fibre such as kenaf (*Hibiscus cannabinus L.*) with approximately 53.4% (w/w) cellulose content (Abdul Khalil et al., 2010) has created considerable interest as a reinforcement filler in polymer composites (Ali et al., 2014; Deka et al., 2013; Holt et al., 2014). Many studies have been conducted in order to investigate the effect of kenaf fibres when these are used as the filler for PLA and the results generally show that a positive reinforcing effect is achieved (Anuar et al., 2012; Bax and Müssig, 2008; Bledzki et al., 2009; Huda et al., 2008; Oksman et al., 2003; Plackett et al., 2003). However, few studies have reported the development of PLA composites with natural fibres for AP applications such as AM packaging materials for food products. In one recent study, Prapruddivongs and Sombatsompop (2012) found that the incorporation of natural fibres such as wood flour into PLA affects the mechanical properties of the resulting films. They reported that the addition of 1.5% (w/w) triclosan AM agent produced only a slight effect on the mechanical properties of neat PLA. However, the inclusion of 10% (w/w) wood flour into the polymeric system was more pronounced than that of the triclosan inclusion. An increase in wood flour content from 0 to 10% (w/w) enhanced the stiffness of the composites and at the same time, reduced the flexibility, tensile strength and toughness.

The incorporation of natural fibres into bio-based plastics together with the addition of naturally-derived AM agents that have minimal impact on the environment is likely to lead to the next generation of packaging materials. The aim of the present study is to investigate the effect of kenaf fibre content and thymol concentration on the mechanical and thermal characteristics of PLA and PLA/kenaf composites.

2. Experimental

2.1. Materials

Poly(lactic acid) (7001D Ingeo$^{\text{TM}}$; specific gravity 1.24; melt flow index (MFI) 6 g/10 min at 210 °C and 2.16 kg; melting temperature range 145–160 °C) was purchased from NatureWorks LLC, USA. Kenaf fibre (bast) was purchased from Ecofibre Industries, Australia. The aspect ratio (L/D) of the kenaf fibres was approximately 9 with an average length of 920 μm and an average diameter of 104 μm. These dimensions were obtained from scanning electron micrograph images. Thymol (T0501) with purity of 99.5% was purchased from Sigma–Aldrich Pty. Ltd., Australia. Sodium hydroxide and acetic acid were purchased from Merck Chemicals, Australia.

2.2. Preparation of neat PLA and PLA composites

Kenaf fibres were soaked in a 5% (w/v) sodium hydroxide solution for 2 h. The fibres were filtered and washed with distilled water before being neutralized by acid treatment upon the addition of a few drops of acetic acid (Amel et al., 2013). The fibres were then filtered, washed and rinsed with distilled water to remove excess acid and neutrality was confirmed using a pH meter. Finally, the fibres were dried overnight in an air circulating oven at 105 °C. Prior to mixing, the PLA resin and fibres were dried in an oven at 60 °C overnight before blending with 5% and 10% (w/w) thymol.

The composites were prepared by melt-blended using an internal mixer (Haake PolyLab OS, Germany) at a fixed screw speed of 50 rpm. The processing temperature and time were set at 155 °C and 8 min respectively and a total mass of 50 g was required to fill the mixing chamber. The mixing torque curves together with the stock temperature (actual temperature of the blend) were simultaneously monitored and recorded using Polysoft OS software during the mixing process. A laboratory press (L0003, IDM Instrument Pty. Ltd., Australia) was used to prepare films (thickness *ca.* 0.3 mm) and slabs (thickness *ca.* 3 mm) for mechanical testing. The materials were preheated at 150 °C for 3 min and pressed at the same temperature for 5 min under a pressure of 50 kN before quench cooling to 30 °C under pressure.

2.3. Composite characterization

2.3.1. Composite morphology

The morphology of the materials was investigated by scanning electron microscopy (SEM). Images of the treated kenaf fibres and tensile fracture surface of neat PLA and PLA/kenaf composites incorporated with thymol were obtained using a JOEL NeoScope (JCM-5000) scanning electron microscope. Samples were mounted on an aluminium sample holder and coated with up to 6 nm of gold using a NeoCoater (MP19020NCTR) under high vacuum and using an accelerating voltage of 10 kV.

2.3.2. Mechanical properties

For each set of composite samples, six tensile specimens were prepared and tested using a Model 4301 Instron Universal Testing Machine with a load cell of 1 kN. Each tensile test was conducted using a cross-head speed of 5 mm min^{-1} on dumbbell-shaped specimens with dimension of 10 × 63 × 3 mm in accordance with the ASTM D638 Type V standard method until tensile failure was detected. The average tensile strength, Young's modulus and percentage extension at break were calculated from the stress–strain curves.

2.3.3. Thermal analysis

Thermal properties of the samples were analyzed using a Mettler Toledo differential scanning calorimetry (DSC) instrument equipped with an intracooler system and under an inert nitrogen gas atmosphere. The thermal properties of the materials were determined and analyzed in accordance with ASTM E1131 standard method using nitrogen gas. Samples of *ca.* 10–12 mg each were weighed and sealed in aluminium crucibles and were then heated from 30 to 300 °C at a rate of 10 °C min^{-1} with a nitrogen flow rate

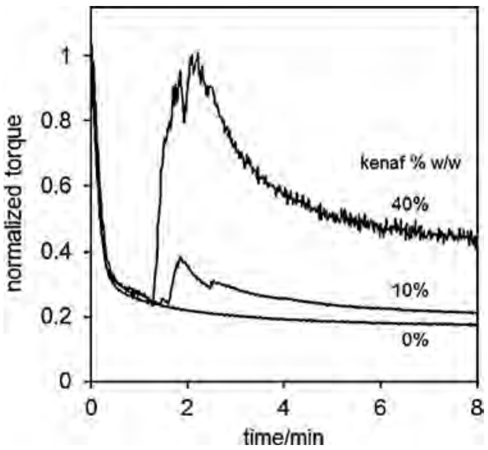

Fig. 1. Normalized torque as a function of time for melts of neat PLA and PLA containing 10% and 40% (w/w) kenaf.

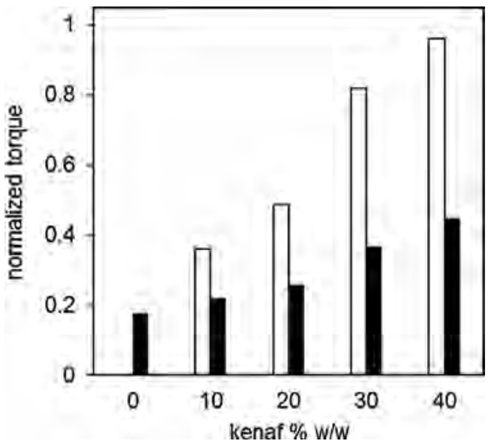

Fig. 2. Normalized secondary torque, $\tau 2$ (□) and equilibrium torque, τeq (■) for melts of neat PLA and PLA/kenaf composites.

of 20 mL min^{-1}. The glass transition temperature (Tg), cold crystallization temperature (Tcc), melting temperature (Tm) and melting enthalpy (ΔHm) were obtained from the DSC thermograms and the percentage of crystallinity (%Xc) of each sample was calculated using equation (1):

$$\%X_c = \frac{\Delta H_m}{\Delta H^{\circ}_m} \times 100 \quad (1)$$

where ΔH°m is the melting enthalpy of 100% crystalline PLA with a value of 93.1 J g^{-1} (Henton et al., 2005). The percentage of crystallinity of neat PLA and PLA/kenaf composites was calculated proportional to the PLA weight percentage in the composites.

A Perkin Elmer TGA 7 was used to obtain the decomposition temperatures of the materials and to determine the approximate weight percentage of thymol that was retained in the samples after processing. The PLA, fibres and composites were heated from 30 to 500 °C at a heating rate of 5 °C min^{-1} and under a nitrogen flow rate of 0.2 L min^{-1}.

3. Results and discussion

3.1. Characterization of the mixing process

The torque value of the formulations during mixing was monitored in order to ascertain that homogeneous mixing had occurred after a sufficient processing time. Upon achieving a consistent torque in the stabilization zone it was deemed that the filler had been well mixed and evenly distributed throughout the matrix (Balakrishna et al., 2013; Premalal et al., 2002). Fig. 1 shows the normalized mixing torque curves of neat PLA and PLA/kenaf composites at zero, 10% and 40% (w/w) kenaf loadings. For neat PLA (Fig. 1 (a)), the initial torque peak or maximum torque ($\tau 1$) was detected at the beginning of processing after which a rapid decrease in the torque occurred before a constant value was attained in the stabilization zone. The $\tau 1$ peak corresponds to the highest mechanical shear forces needed to cause the cold PLA resin to flow. As the PLA becomes completely molten, the torque rapidly decreases. For each of the PLA/kenaf composite formulations, the $\tau 1$ peak was also observed during processing and the intensity of this peak decreased as the fibre content increased due to the concomitant decrease in the amount of PLA in the formulation. After the initial mixing, a secondary torque peak ($\tau 2$) was recorded in each melt after approximately 2 min of mixing and this peak is due to the presence of the fibres in the melt.

Fig. 2 shows the normalized $\tau 2$ and equilibrium torque (τeq) of PLA/kenaf formulations at various kenaf contents ranging from zero to 40% (w/w). The results show that as the kenaf content is increased, the $\tau 2$ and τeq values increase almost linearly where the PLA/kenaf composite containing 40% (w/w) kenaf exhibits the highest secondary and equilibrium torque values of any of the systems that were tested. The increase in $\tau 2$ with increasing kenaf content is attributed to the increase in mechanical shear that is required to mix larger amounts of kenaf into smaller amounts of molten polymer. This occurs towards the end of the processing period where the equilibrium torque is observed, the latter of which is also dependent on the kenaf content.

Fig. 3 shows the normalized torque curve for neat PLA and those curves for neat PLA to which 5% or 10% (w/w) thymol was added after approximately 2 min in the mixer. The addition of thymol causes the torque to decrease momentarily but eventually the equilibrium torque is achieved as soon as the thymol is mixed throughout the melt. The observed decrease in the torque upon addition of thymol suggests that the additive acts as a lubricant or plasticizer. The behaviour of thymol as a plasticizer in the system is confirmed by the final normalized torque readings in the stabilization zone (see inset in Fig. 3). These normalized τeq values were found to be 0.16 and 0.10 for the systems containing 5% and 10% (w/w) thymol respectively compared to the neat PLA that produced a normalized τeq of 0.18.

Fig. 4 depicts the normalized torque curves for PLA composites with 40% (w/w) kenaf containing zero, 5% or 10% (w/w) thymol.

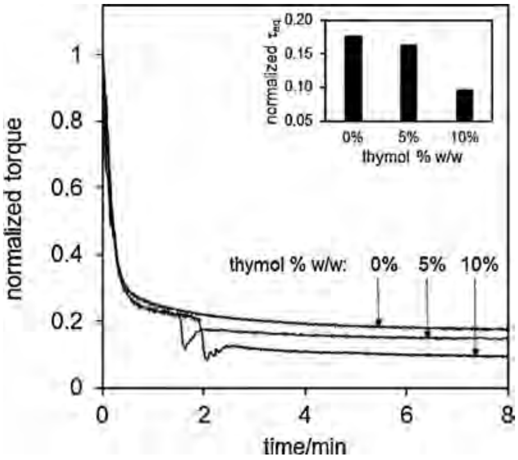

Fig. 3. Normalized torque as a function of time for melts of PLA containing zero, 5% and 10% (w/w) thymol. Inset: corresponding normalized equilibrium torque for each of these systems.

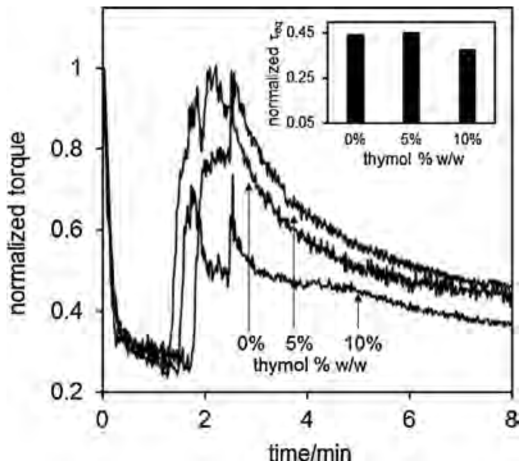

Fig. 4. Normalized torque as a function of time for melts of PLA/kenaf composite (40%, w/w, kenaf) containing zero, 5% and 10% (w/w) thymol. Inset: corresponding normalized equilibrium torque for each of these systems.

The addition of 10% (w/w) thymol into the melt after 2 min has a significant effect on the rheology as the $\tau 2$ and τeq values for this formulation were found to be significantly lower than their counterparts in the other two formulations. It can therefore be suggested that by increasing the thymol concentration in the melt, one can reduce the mechanical shear forces due to the plasticizing and mixing effect of this AM agent. A similar finding was observed by Xue et al. (2008) who studied the effect of water content on the rheological behaviour of starch. In the case of the latter study it was found that the torque decreased with increasing water content.

3.2. Fracture surface imaging

The SEM image of neat PLA shown in Fig. 5(a) reveals a relatively smooth surface in which there is evidence of boundaries presumably created upon the stress fracture due to the brittleness of the PLA. The inherently brittle nature of PLA (Mukherjee and Kao, 2011) is confirmed by the large cracks that appear on the surface. The addition of 10% (w/w) thymol to PLA results in reduced surface cracking (see Fig. 5(b)) and this is also consistent with the notion that thymol behaves as a plasticizing agent in the system (see also Fig. 3).

Fig. 5(c) and (d) shows SEM images of a tensile fracture surface of PLA formulations that contain 40% (w/w) kenaf. It can be seen that in each of these composites the kenaf fibres are well dispersed throughout the polymer matrix. In the case of the composite containing no thymol (Fig. 5(c)) the kenaf fibres appear to be more tightly bound to the matrix compared to the composite in which thymol is present (Fig. 5(d)). In the latter case the presence of protruding fibres at the surface suggests that the thymol may be more concentrated at the interface between the fibres and the polymer matrix thereby acting as a lubricant that facilitates the slippage of the fibres out of the matrix during fracture. This behaviour is also consistent with the lower equilibrium torque observed in the composite melts containing thymol compared to those where no thymol was present (see Fig. 4). The smooth surfaces of some of the fibres together with the voids that can be seen in Fig. 5(d) also suggests that there is weak adhesion between the fibres and the PLA matrix when thymol is present in the formulation.

3.3. Tensile properties

The effect of incorporating different amounts of kenaf on the tensile strength of the PLA formulations is shown in Fig. 6 where the normalized tensile strength (TS), Young's modulus (*E*) and percent elongation at break (ε) are given for systems containing various kenaf loadings and zero, 5% and 10% (w/w) thymol.

3.3.1. Tensile strength

In Fig. 6(a) it is evident that at kenaf loadings from 10–30% (w/w), the tensile strength of the PLA/kenaf formulations containing zero and 5% (w/w) thymol is slightly increased by up to *ca.* 11% and 14% respectively compared with the neat PLA. This suggests

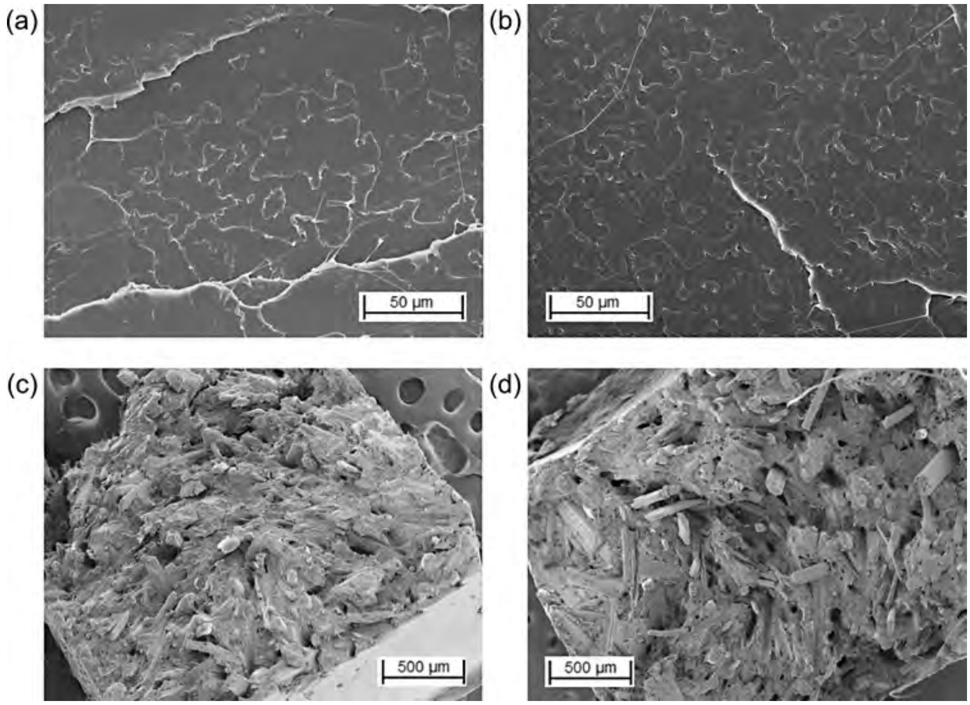

Fig. 5. Scanning electron micrograph images at 500× magnification of: (a) neat PLA and (b) PLA containing 10% (w/w) thymol; and images at 50× magnification of: (c) PLA containing 40% (w/w) kenaf and (d) PLA containing 40% (w/w) kenaf and 10% (w/w) thymol.

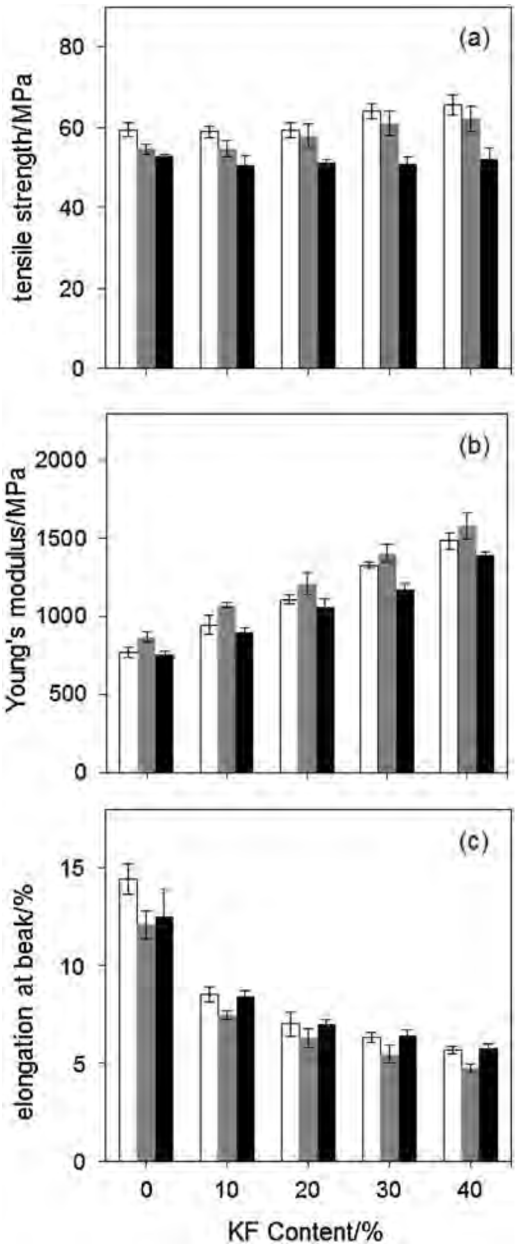

Fig. 6. Tensile properties of PLA composites containing: (□) zero, (■) 5% (w/w) and (■) 10% (w/w) thymol: (a) tensile strength (TS); (b) Young's modulus (*E*); and (c) percent elongation at break (*ε*).

that the kenaf fibres act not only to fill the PLA but also provide reinforcement for the composite. This reinforcement may occur through a mechanism in which the applied stress is transferred from one fibre to the next thereby enabling an even distribution of the stress throughout the material (Avella et al., 2009). The rough surface topography of the kenaf fibre as apparent in the SEM image (see Fig. 7) may increase the adhesive characteristics and thus facilitate the stress transfer during an applied load due to the good mechanical interlocking between the kenaf fibres and the matrix. The rough surface of kenaf fibre results from the removal of some lignin, pectin, hemicellulose as well as unwanted impurities from the surface during the alkali treatment of the fibres. Similar findings have been reported by Ibrahim et al. (2010) who incorporated 10–30% (w/w) kenaf fibre into a PLA matrix. They reported that the tensile strength of the composite increased with an increase in the amount of the fibre in the composite. However, the tensile strength of their composite materials dropped at above 30% (w/w) kenaf content. They speculated that the amount of matrix is probably insufficient to wet out the fibre and fully transfer the stress effectively at such high fibre loadings. Furthermore, this may also be due to high fibre–fibre interaction and agglomeration of fibres in the PLA matrix. Nevertheless, the latter result is in agreement with the current findings whereby the tensile strength exhibited a plateau from 30 to 40% (w/w) of kenaf loading. This suggests that the fibres were well distributed and had a sufficient extent of interaction with the PLA matrix.

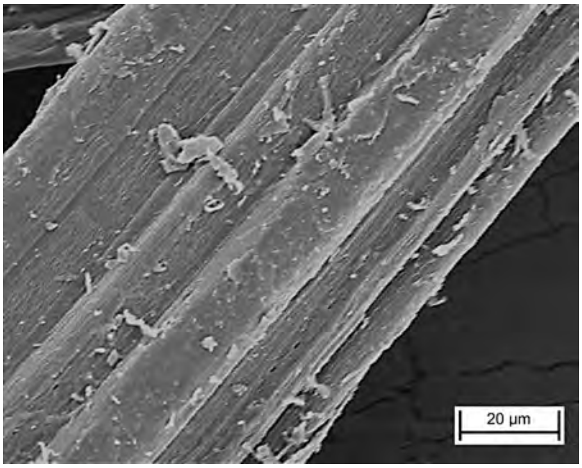

Fig. 7. Scanning electron micrograph image of kenaf fibre at 20× magnification.

No significant difference between the tensile strength of the PLA/kenaf formulations without thymol or those containing 5% (w/w) thymol was observed across the range of kenaf loadings that were tested. This may be due the level of thymol being too small to affect any change in the tensile strength that could be reliably detected given the inherently low sensitivity of tensile testing as an experimental technique in such cases. Thus any changes in the tensile strength that may arise due to the plasticizing effect of the thymol in the formulation may not be seen at such low levels of thymol. Furthermore, a small amount of the thymol might have been lost from the formulation as a result of the thermal processing, the effect of which may be more critical at low levels of thymol.

Similarly, no significant change in the tensile strength of the PLA/kenaf formulations containing 10% (w/w) thymol was observed with increased kenaf content over the range of formulations prepared in the study (see Fig. 6(a)). For these formulations the average tensile strength was found to be *ca.* 50 MPa with 60 MPa for neat PLA. The inclusion of thymol at the higher level of 10% (w/w) compared to the other series of formulations has a measurable effect on the tensile strength and it appears to negate the increase in tensile strength that would normally be imparted by the kenaf fibres in the absence of thymol. This may again be attributed to the plasticizing effect of thymol within the PLA matrix and is consistent with the observations made from the micrograph images of the tensile fracture surface (Fig. 5). In particular, the thymol may interfere with the interaction and/or stress transfer between the PLA matrix and the kenaf fibres during the applied stress. As such, this disturbance of stress transfer is likely to facilitate the end slip of fibres from the polymer matrix, particularly in the presence of the plasticizing agent (see also Fig. 5(d)).

Kramer (2009) reported a decrease in the tensile strength of thermoplastic starch films upon coating with AM additives and it has been suggested that the interactions between the AM additives and the polymeric matrix affects the intermolecular forces within the matrix in a similar manner to water acting as a plasticizer (Chabrat et al., 2012). Taib et al. (2009) studied the effect of the plasticizer poly(ethylene glycol) (PEG) on the tensile strength of PLA/kenaf composites and found that a plasticized PLA/kenaf composite had a lower tensile strength than that of an un-plasticized composite at 40% (w/w) kenaf content. Moreover, the effect of thymol addition observed in the present study is in agreement with the findings of Prapruddivongs and Sombatsompop (2012) who found that an addition of 0.5–1.5% (w/w) triclosan into PLA composites containing 10% (w/w) wood flour resulted in a reduction in the tensile strength of the composites from 45 to 40 MPa.

3.3.2. *Young's modulus*

Fig. 6(b) shows the effect of various kenaf loadings on the Young's modulus of PLA composites containing zero, 5% and 10% (w/w) thymol. Overall, these results suggest an almost linear increase in the Young's modulus with the addition of kenaf fibre at all concentrations of thymol for which the composite containing 40% (w/w) kenaf showed the highest stiffness at approximately 1500 MPa. This may be due to the kenaf fibres imparting an overall increase in stiffness to the composite as the kenaf content is increased. This is in agreement with the findings of Avella et al. (2009) who suggested that the modulus of the fibre component is the dominant contributor to the tensile modulus of the composite. A general upward trend in the Young's modulus with increased filler content in the composite was also observed in the case of polypropylene (PP)/wood fibre composites where the stiffness of the composite was found to be dependent on the filler content and homogeneity of the filler dispersion (Karmarkar et al., 2007). These findings are also in agreement with a study of PLA/wood flour/triclosan composites where an increase in wood flour from 0 to 10% (w/w) enhanced the stiffness and concurrently reduced the flexibility and toughness (2012). In the present study, the plasticizing effect of the thymol that was observed in the results of the tensile tests in Fig. 6(a) is also reflected in the Young's modulus data in Fig. 6(b) particularly at the higher kenaf loadings. For example, at kenaf loadings greater than 20% (w/w), a concomitant decrease in the Young's modulus that occurs upon an increase in thymol concentration can be clearly seen. Indeed, the addition of 10% (w/w) thymol to the composite formulation in general results in a marked reduction in stiffness compared to the composites without thymol and is consistent with the notion that the thymol may be concentrated at the interface between the kenaf fibres and the polymer matrix as discussed in relation to Fig. 5(d). A decrease in the Young's modulus of PP films impregnated with 8% (w/w) thymol supports the present findings (Ramos et al., 2012). It can therefore be suggested that the addition of thymol into PLA/kenaf composites decreases the stiffness of the materials due to the plasticizing effect of the AM agent.

3.3.3. *Elongation at break*

The effect of various kenaf loadings on the elongation at break of the PLA composites containing zero, 5% and 10% (w/w) thymol is shown in Fig. 6(c). As expected, the elongation at break of these composites generally decreases with increasing amounts of kenaf. This suggests that the presence of the kenaf fibres does not contribute to the elasticity or the final composite flexibility. The observed decrease in flexibility of the composites is likely to be related to the high stiffness of the composites as is apparent in Fig. 6(b). The effect of thymol addition on the elongation at break of the composites can also be seen in Fig. 6(c). The PLA/kenaf composites containing 10% (w/w) thymol exhibited slightly higher values of the elongation at break compared with the other formulations in the series. This increase may be due to the AM agent causing a decrease in the compatibility between the PLA matrix and the filler in the composite system or the possible slippage effect as evidenced in the SEM images above (see Fig. 5(d)). According to Abdul Khalil et al. (2012b), poor fibre-matrix bonding decreases the strength, stiffness, interfacial adhesion and increases the flexibility of a composite due to the capability of the filler particles to split and fall apart. In the present study, the kenaf fibres may have become less stiff in the presence of thymol, which is a volatile essential oil extract. In the case of non-volatile agents such as nisaplin and EDTA incorporated into plasticized PLA/glycerol triacetate films, a significant reduction of the elongation at break from 108.5 to 62.5% and the impact strength from 5.4 to 3.4 J mL^{-1} in comparison with the plasticized PLA has been reported (Liu et al., 2010).

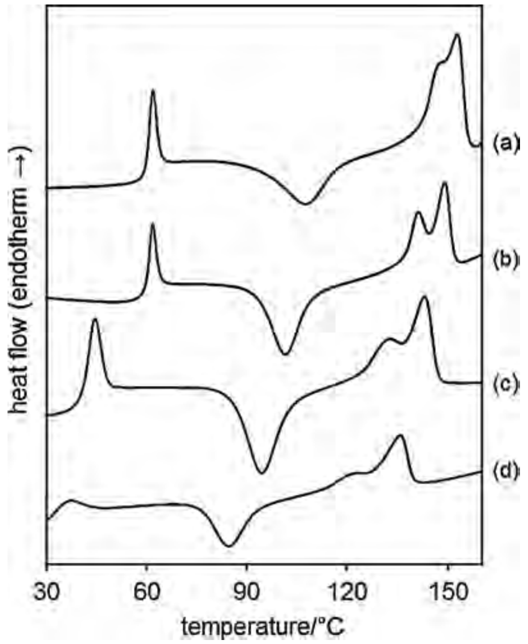

Fig. 8. DSC thermograms of (a) neat PLA and PLA containing: (b) 40% (w/w) kenaf, (c) 10% (w/w) thymol and (d) 40% (w/w) kenaf and 10% (w/w) thymol.

3.4. *Thermal properties*

3.4.1. *Differential scanning calorimetric analysis*

The thermal properties of composite samples were studied by DSC analysis. The DSC thermograms of neat PLA and PLA composites are shown in Fig. 8. Neat PLA exhibited a *Tg* peak at 61.7 °C and *Tcc* peak at 107.8 °C (see Fig. 8(a)). The peak melting temperature, *Tm*, was observed to be 152.5 °C which is in the typical range for a semi-crystalline polymer (Byun et al., 2010).

The values of *Tg*, *Tcc*, *Tm*, Δ*Hm* and %*Xc*, of the various formulations were determined from the complete DSC analysis and these are summarized in Table 1. It was found that the incorporation of kenaf at 10% or 40% (w/w) (Fig. 8(b)) into the PLA matrix resulted in no significant change to the *Tg*, whereas a slight increase in the *Tg* seemed to occur at a 10% (w/w) loading of kenaf (see Table 1). These results seem contrary to the findings of Rahman et al. (2012) who reported that the *Tg* of PLA filled with natural fibres demonstrated a decreasing trend in *Tg* with increasing fibre content. It remains unclear as to why such a trend was not observed in the current study. Nonetheless, the *Tcc* peak of PLA that has a high content of fibre (40% (w/w) kenaf) is observed at a lower temperature (101.7 °C) compared to neat PLA (107.8 °C). This effect has been previously reported for composites of PLA/kenaf and PP/kenaf and was attributed to the kenaf fibres acting as a nucleating agent (Han et al., 2012).

The incorporation of 10% (w/w) thymol in neat PLA and in the PLA/kenaf composites resulted in a decrease in *Tg* and *Tcc* (see Fig. 8(c) and (d) as well as Table 1). This may be due to the plasticizing effect of thymol that lowers the *Tg* in particular and this

Table 1
Thermal analysis values obtained from DSC thermograms of neat PLA, PLA/kenaf and PLA/kenaf/thymol composites.

Kenaf content/% (w/w)	Thymol content/% (w/w)	T_g/°C	T_{cc}/°C	T_{m1}/°C	T_{m2}/°C	ΔH_m/J g^{-1}	X_c/%
0	0	61.7	107.8	147.5[a]	152.5	26.1	28.0
	5	53.0	101.3	139.6	147.2	24.6	27.8
	10	44.5	94.5	132.6	142.7	22.1	26.4
10	0	62.2	107.7	146.1	152.4	23.3	27.8
	5	52.5	98.7	137.6	147.2	19.9	27.6
	10	39.1	90.6	128.7	140.4	16.6	26.2
40	0	61.7	101.7	141.0	148.8	15.4	27.6
	5	50.4	91.9	132.7	143.1	13.5	26.3
	10	37.4	84.5	113.8	135.5	12.2	26.1

[a] Shoulder on the main PLA peak.

observation is consistent with the rheology and tensile properties results that were obtained for neat PLA and the PLA/kenaf composites containing thymol (see Figs. 2, 3 and 6). Similar findings have been reported by Prapruddivongs and Sombatsompop (2012) whereby the addition of 1.5% (w/w) triclosan decreased the *Tg* of neat PLA and PLA containing 10% (w/w) wood flour.

In the present study, however, the neat PLA containing 10% (w/w) thymol demonstrated a narrower *Tg* peak than the PLA/kenaf composite containing 10% (w/w) thymol (see Fig. 8(c) and (d)) and this may be due to the lower amount of PLA present in the composite material. Fig. 8(c) also shows that the *Tcc* peak narrowed with the addition of 10% (w/w) thymol in PLA and shifted to a lower temperature. A similar finding has been reported by Byun et al. (2010). Nevertheless, the addition of thymol to the PLA/kenaf composites containing 40% (w/w) kenaf resulted in a lower *Tcc* with a slightly broader peak (Fig. 8(d)).

The neat PLA exhibited what can be considered to be a single melting peak at 152.2 °C with a possible shoulder at *ca.* 147.5 °C whereas two distinct melting peaks were observed for all of the PLA/kenaf composites. Both melting peaks of the composites shifted to lower temperatures as the fibre loading was increased. This behaviour is consistent with other studies where it has been reported that an increase in the fibre content results in the appearance of a small, secondary melting peak which shifts to lower temperatures (Prapruddivongs and Sombatsompop, 2011). The minor peak may be due to either a different PLA crystalline phase that forms only in presence of the fibres and/or melting of the transcrystalline zone (Prapruddivongs and Sombatsompop, 2012).

The addition of higher loadings of kenaf also decreased the peak *T*m temperatures and the Δ*H*m of PLA across the range of systems that were studied. For example, at zero thymol concentration the *T*m decreases from 152.5 °C (pure PLA) to 141.0 °C (*T*m1 at 40% (w/w) loading kenaf). The Δ*H*m for these corresponding samples decreased from 26.1 to 15.4 J g^{-1} respectively with no significant changes on the percentage of crystallinity, *Xc*. Byun et al. (2010) also observed a decrease in the T_m of PLA films with addition of PEG.

It may be expected that the addition of fillers or additives such as kenaf fibre or thymol could result in the appearance of a second *T*m peak in the DSC thermogram where the presence of the additive causes a secondary crystalline phase at the boundary of the bulk polymer and the additive. For example, in the present study, two melting peaks are observed at 132.6 and 142.7 °C for PLA containing 10% (w/w) thymol with zero kenaf loading. Both of these *T*m peaks appear at correspondingly lower temperatures than those of the PLA/kenaf composite containing 40% (w/w) kenaf loading but without thymol which appear at 141.0 and 148.8 °C (see Table 1). This observation is in agreement with the other research findings where a minor secondary melting peak appeared in a PLA film formulation containing a plasticizer such as PEG (Byun et al., 2010). Furthermore, a weaker secondary *T*m peak is observed in the PLA composite containing both kenaf (40%, w/w) and thymol (10%, w/w) and both of these melting peaks appear at lower temperatures than the corresponding peaks in the formulations that have only one of the two additives (see Fig. 8(d)). This may also be due to the lower amount of PLA in the PLA/kenaf composite system. Interestingly, the *Xc* values for the systems containing thymol show no significant difference (see Table 1).

3.4.2. Thermogravimetric analysis

Fig. 9 shows the thermogravimetric analysis (TGA) profiles of neat PLA and PLA/kenaf composites containing 10 and 40% (w/w) kenaf in the form of the normalized weight percentage as a function of temperature. There is a small but noticeable step in the first stages of the profiles of the composites that is due to the presence of water in the kenaf fibres and this step is more evident in the profile of the composite containing 40% (w/w) kenaf. A single degradation step was observed for neat PLA with no char residue apparent at temperatures above *ca.* 380 °C. The rate of weight loss of neat PLA passes through a maximum in the degradation temperature range between 280 °C and 355 °C with the maximum occurring at 333 °C (see Fig. 9(b)). As expected, the addition of kenaf fibres to the

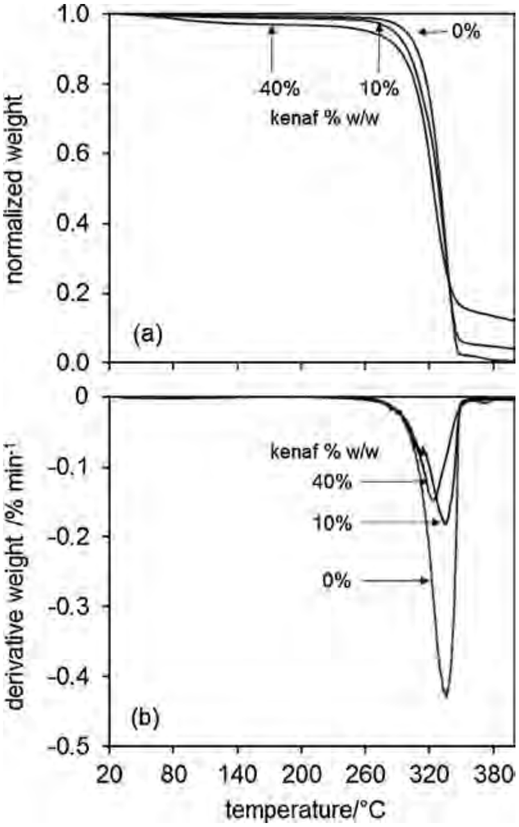

Fig. 9. Thermogravimetric profiles of (a) PLA containing zero, 10% and 40% (w/w) kenaf; and (b) first derivatives of these systems.

composite decreases the temperature at which the onset of PLA degradation occurs. This is also reflected in the derivative curves where the maximum rate of degradation for the neat PLA and PLA containing 10% (w/w) and 40% (w/w) kenaf occurs at the temperatures of 333, 332 and 321 °C respectively. The third degradation temperature of the composite containing 40% kenaf content was observed at approximately 370 °C with a small but noticeable peak. This may be due to the degradation of a non-cellulosic substance such as lignin, waxy material or sugar mallards (Bourmaud et al., 2010). Generally, a lower degradation temperature is observed for plant fibres alone under nitrogen gas as compared to air (Van de Velde and Baetens, 2001). However, the fibre degradation peak would be expected to be more pronounced under an air atmosphere (Bourmaud et al., 2010).

The degradation temperature of PLA/kenaf composites therefore demonstrates a decreasing trend with increasing kenaf content. This occurs due to some portions of the PLA matrix having been replaced with the less thermally stable kenaf fibres that reduce the thermal stability of the polymer matrix as a whole (Rahman et al., 2012; Yussuf et al., 2010). This result is in agreement with the findings of Yussuf et al. (2010) who investigated the thermal stability of neat PLA and PLA/kenaf composite samples containing 20% (w/w) kenaf using TGA. They observed that the composite degraded at 321 °C compared with neat PLA which degraded at 323 °C at a weight loss of 10%. In addition, the latter composites also showed obvious char residues at the end of the degradation process and this is consistent with the data shown in Fig. 8. In the present study the char residues of composites containing 40% and 10% (w/w) kenaf were 10% and 4% respectively at a degradation temperature of 380 °C. It is interesting to note that the presence of cellulose in the kenaf fibres, which is a lignocellulosic material, results in the production of char residue which, according to Mitra et al. (1998), may improve the fire retardancy of the material.

The TGA profiles of neat PLA and PLA containing 5% and 10% (w/w) of thymol are shown in Fig. 10(a). The profile of the neat PLA exhibits a single step across the temperature range of *ca.* 30–400 °C whereas the profile of the formulations containing thymol exhibits two main steps during the analysis. In the case of the latter, the first of these steps occurs gradually over the temperature range of *ca.* 30–300 °C and is attributable to the evaporation and/or loss of thymol from the polymer matrix. This observation is in agreement with the work of Ramos et al. (2012) who investigated by TGA the retention of AM agents such as thymol and/or carvacrol in a PP matrix after processing. In that study it was suggested that the evaporation and/or volatilization of thymol from the polymer matrix commenced at a specific temperature within the range of 100–200 °C and not immediately after the temperature was increased during the TG analysis. This behaviour can also be seen in Fig. 10. The second step observed in the profile of the thymol-containing formulations (see Fig. 10) occurs over the temperature range of *ca.* 310–330 °C and is attributable to the almost complete degradation of the polymer matrix as evidenced by the absence of a persistent char at high temperatures.

Fig. 10(b) shows the TGA profiles of PLA composites containing 40% (w/w) kenaf and containing thymol at zero, 5% and 10% (w/w). These profiles relate directly to the profiles of the PLA formulations containing kenaf alone (see Fig. 9) as well as those containing thymol alone (see Fig. 10) in that a single degradation step is observed over the temperature range from *ca.* 30–200 °C. This is followed by a second step due to the thermal degradation of the polymer matrix as well as there being evidence of a char residue at higher temperatures. The initial weight loss observed in the temperature range of *ca.* 30–100 °C for the PLA/kenaf composite without thymol in Fig. 10 is due to the evaporation of water that is present in the kenaf fibres (see also Fig. 9) whereas the initial weight loss of those composites containing thymol is due to the evaporation of water as well as the degradation and/or evaporation of thymol. The latter predominantly occurs in the temperature range of 100–200 °C.

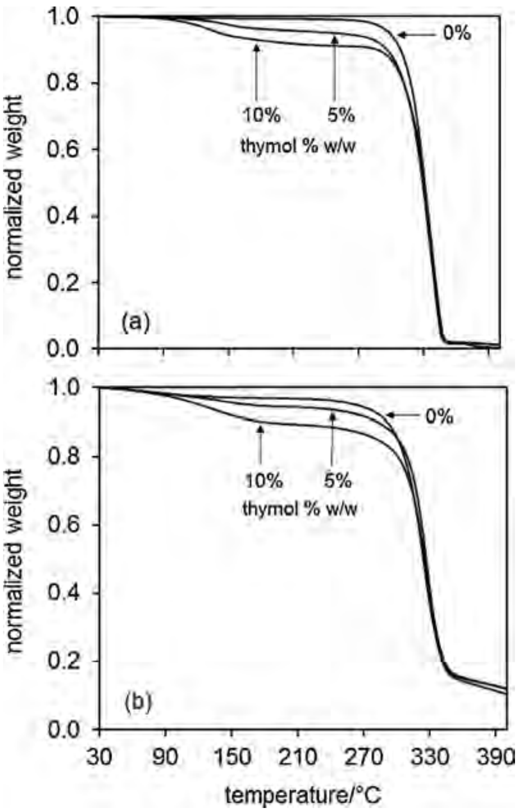

Fig. 10. Thermogravimetric profiles of (a) PLA containing zero, 5% and 10% (w/w) thymol; and (b) PLA/kenaf composite (40%, w/w, kenaf) containing zero, 5% and 10% (w/w) thymol.

It is also clear that the higher the kenaf content in the composite, the higher will be the moisture content. The affinity of the kenaf fibres for water may be due to the alkali surface treatment that leads to an increase in the extent of surface roughness as well as the hydroxyl group accessibility (Karmakar, 1999; Vilaseca et al., 2007). From the data presented in Fig. 10(b), it can be determined that the PLA/kenaf composites that were formulated with 5% and 10% (w/w) thymol retain about 3% and 7% (w/w) of the thymol that was originally added to the formulation respectively. Thus there has been some loss of the volatile AM agent during processing (Ramos et al., 2012). The moisture that is present within the kenaf fibres may also facilitate the loss of the AM agent.

It can be seen from the weight loss curves in Fig. 10(a) and (b) that the loss of thymol from the composites containing kenaf started right at the beginning of the heating process as compared to the loss of thymol from the neat PLA where a delay is observed. This immediate loss of thymol from the composite containing kenaf may be due partially to the ends of the kenaf fibres facilitating the escape of the thymol. This is consistent with the micrograph images in Fig. 5 where PLA containing 40% (w/w) kenaf and 10% (w/w) thymol that show the presence of voids in the facture surface that were possibly created by the fibres in the composite.

4. Conclusions

The characterization of PLA and PLA composites was successfully performed using mechanical and thermal analytical techniques in order to investigate the effects of the natural filler kenaf as well as the AM additive thymol on the stability and performance of the material during processing.

The results suggest that the addition of thymol to PLA and PLA/kenaf composites imparts a plasticizing effect that modifies the rheology and subsequent mechanical properties of the processed materials. In particular, the equilibrium torque value of each of the materials was decreased during processing with the addition of thymol thereby offsetting, to some extent, the effects of the incompatibility between the PLA matrix and the kenaf fibres. This observation was supported by evidence obtained from high magnification images of the fracture surface of the PLA/kenaf composites.

The incorporation of thymol into PLA/kenaf composites also decreased the tensile strength with no significant change observed upon increasing the kenaf fibre loading. The tensile strength and stiffness of PLA/kenaf composites however increased with higher fibre loadings thereby imparting a reinforcement effect within the PLA composite. Moreover, it was found that an acceptable tensile strength was attained by the PLA/kenaf composites containing thymol compared to the commercial neat PLA thereby rendering these materials as potential candidates for commercial packaging systems. Nonetheless, the effect of AM agent addition on the impact strength of the material might also be an interesting property to investigate prior to developing these systems for commercial applications.

The DSC analyses showed a general decrease of *T*g, *T*cc, *T*m and ΔHm, with no significant changes in %*X*c, for PLA and PLA/kenaf composites containing 5% and 10% (w/w) thymol. It was noted that an increased loading of kenaf fibres in the PLA resulted in a decrease in the TGA decomposition temperature indicating a reduced thermal stability of the PLA. The TGA results also suggested that thymol can be released from the PLA/kenaf matrix at relatively low temperatures and as such has the potential to impart AM activity. Further work in our laboratory is currently underway to evaluate the kinetics of thymol release from these systems and their subsequent AM activity in order to develop effective active food packaging materials.

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Two Novel Algorithms for the Thermogravimetric Assessment of Polymer Degradation under Non-Isothermal Conditions

Overview

This standalone chapter provides an insight into polymer degradation under non-isothermal conditions as presented by two novel algorithms. The algorithms were validated using model data and applied to thermogravimetric (TG) data obtained during the degradation of PLA and PLA/kenaf composites containing thymol under non-isothermal conditions.

The paper entitled “Two Novel Algorithms for the Thermogravimetric Assessment of Polymer Degradation under Non-Isothermal Conditions” by Bigger S. W., Cran M. J. and Tawakkal I. S. M. A. was published in *Polymer Testing,* **43**, 139-146.

PART B:

DECLARATION OF CO-AUTHORSHIP AND CO-CONTRIBUTION: PAPERS INCORPORATED IN THESIS BY PUBLICATION

This declaration is to be completed for each conjointly authored publication and placed at the beginning of the thesis chapter in which the publication appears.

Declaration by [candidate name]: **Signature:** [redacted] **Date:** 02.03.2015

Intan S. M. A. Tawakkal

Paper Title:

Two Novel Algorithms for the Thermogravimetric Assessment of Polymer Degradation under Non-Isothermal Conditions

In the case of the above publication, the following authors contributed to the work as follows:

Name	Contribution %	Nature of Contribution
Stephen W. Bigger	70	Prepared major part of the manuscript Contribution to writing of paper
Marlene J. Cran	20	Contribution to writing of paper and journal submission
Intan S. M. A. Tawakkal	10	Designed the experiment Performed the sample analyses

DECLARATION BY CO-AUTHORS

The undersigned certify that:

1. They meet criteria for authorship in that they have participated in the conception, execution or interpretation of at least that part of the publication in their field of expertise;
2. They take public responsibility for their part of the publication, except for the responsible author who accepts overall responsibility for the publication;
3. There are no other authors of the publication according to these criteria;
4. Potential conflicts of interest have been disclosed to **a)** granting bodies, **b)** the editor or publisher of journals or other publications, and **c)** the head of the responsible academic unit; and
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Property modelling

Two novel algorithms for the thermogravimetric assessment of polymer degradation under non-isothermal conditions

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Two novel algorithms are presented for processing thermogravimetric (TG) data obtained during the degradation of a polymer in a single step mechanism under non-isothermal conditions. The first algorithm assesses three characteristics computed from the TG profile against a theoretical data set, and identifies likely kinetic models to fit the experimental data. The second algorithm provides an iterative arithmetic method to extract the apparent activation energy, E_a, and Arrhenius A-factor, A, from TG data without simplifying assumptions. The algorithms are validated using model data and applied to data for the non-isothermal degradation of poly(ethylene adipate), poly(lactic acid) (PLA) and a food packaging PLA composite formulation containing kenaf, a natural fibre. The analysis of poly(ethylene adipate) produced $E_a = 137$ kJ mol^{-1} and $\log_{10}A = 8.71$ (first-order kinetic model). The kenaf fibre destabilizes PLA, lowering its E_a from 190 kJ mol^{-1} to 150 kJ mol^{-1} (contracting volume model).

1. Introduction

The scientific literature on the application of thermogravimetric analysis (TGA) and differential thermal analysis (DTA) techniques experienced a huge expansion in the decade following the late 1960's [1]. These techniques have since been used as valuable tools to investigate synthetic polymer degradation as well as a wide range of other materials, including cellulose [2,3] and various solids such as carbonates [4–6] and active solids [7]. Early reviews in the area include the work of Mitchell and Chiu [8–11] and Murphy [12,13]. More recently, Vyazovkin et al. [14] published a comprehensive account of methods to evaluate kinetic parameters such as the activation energy, Arrhenius A-factor and the reaction model, from data obtained *via* TGA, DTA and differential scanning calorimetry (DSC) experiments. The work covers the most common kinetic methods, including the model-free (iso-conversional) ones as well as model fitting.

The application of TGA to the study of polymer degradation has been primarily focused on deriving kinetic parameters, such as the apparent activation energy and Arrhenius A-factor, that in some cases may be used as indicators of thermal stability. To obtain these parameters, TGA experiments can be performed either isothermally at a series of constant temperatures or non-isothermally under a known, usually linear, temperature ramp at a number of different heating rates. Clearly, the major advantage of the latter over the former is that less time is usually required to experimentally obtain the data for analysis. Nonetheless, criticisms that are often leveled at non-isothermal methods include: (i) the fact that the mechanism, kinetic model, and hence the kinetic parameters, may change with temperature over the course of the experiment, (ii) over extreme temperature ranges, and particularly under oxidizing conditions, the material at the end of the experiment bears little chemical resemblance to the starting material and (iii)

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at high heating rates there may be thermal lag effects [1,14,15].

In order to extract kinetic parameters from experimentally acquired TGA data, numerous kinetic models and methods of mathematically processing the data in accordance with the proposed model have been suggested. Early papers by Dollimore et al. [16,17] present numerical criteria derived from theoretical modeling that can be used to help identify suitable kinetic models that may be applied in fitting a given set of data. The mathematical methods [18,19] for processing thermogravimetric (TG) data in accordance with a given model fall into two main categories, namely the integral methods [14,20] and differential methods. The respective merits of these are extensively discussed in the literature [14,15,21].

Many of the earlier studies in this field describe ways in which experimental data can be analyzed in accordance with an appropriate kinetic model, given that the required mathematical manipulations are often very difficult. Indeed, some of the early methods for processing experimental TGA results can only be described as tedious and cumbersome at best, and alternative methods were widely explored in an attempt to simplify the procedures. However, simplifying assumptions and approximations may have a serious effect on the outcome.

Notwithstanding the extreme caution one must use in applying the results derived from TGA studies, particularly to the prediction of polymer service lifetimes, the technique can provide in some cases a useful basis on which to compare the apparent stability of polymeric materials. Such comparative data can be used in conjunction with other stability information in order to provide a more complete understanding of the overall stability of the material. In view of the potentially useful information that can be derived from TGA experiments, and the advent of modern computer-based iterative numerical methods that can overcome many of the previously encountered difficulties, or indeed impossibilities, associated with solving complex systems of equations that do not conform to closure, a computer-based algorithmic procedure was developed for: (i) the analysis of non-isothermal TGA data and (ii) the verification that the chosen analysis is appropriate. Indeed, an integral numerical approach to solving isoconversional systems has been heralded by Vyazovkin and Dollimore [22] and Vyazovkin [23,24] as a means by which kinetic parameters can be more accurately determined from experimental TG data.

2. Theory

The recent review by Vyazovkin et al. [14] provides the basic theory and recommendations for performing computations on data acquired from thermal analytical methods. The following is intended as a brief overview of the basic theoretical principles that apply directly to the approach that is described in the current work.

2.1. Kinetic equation for non-isothermal TGA

For the case of non-isothermal TGA, the following equation applies and is derived in the Supplementary Material:

$$g(\alpha) = (AE_a/R\beta)\int_x^{\infty}[\exp(-x)/x^2]\mathrm{d}x \tag{1}$$

where $g(\alpha) = 1/\mathrm{d}f(\alpha)/\mathrm{d}\alpha$, α is the extent of the reaction (conversion), A is the Arrhenius A-factor, E_a is the apparent activation energy, R is the ideal gas constant, β is the heating rate and $x = E_a/RT$. The function $f(\alpha)$ describes the kinetics of the system. Eq. (1) can be written more simply as:

$$g(\alpha) = (AE_a/R\beta) \times \mathrm{p}(x) \tag{2}$$

where the function $\mathrm{p}(x)$ represents the integral $\mathrm{p}(x) = \int_x^{\infty}[\exp(-x)/x^2]\mathrm{d}x$

2.2. Differential and isoconversional data analysis equations

For a given process where (T, α) data exist and the kinetic form of $f(\alpha)$ along with the heating rate, β, are known, the following equation applies:

$$\ln[(\mathrm{d}\alpha/\mathrm{d}T)/f(\alpha)] = \mathrm{In}(A/\beta) - E_a/RT \tag{3}$$

The application of this equation is representative of one of the so-called "differential" methods for analyzing TGA data, and the derivation of eq. (3) is given in the Supplementary Material. A plot of $\ln[(\mathrm{d}\alpha/\mathrm{d}T)/f(\alpha)]$ versus $1/T$ will enable the value of the apparent activation energy, E_a, to be calculated from the gradient of the plot. The Arrhenius A-Factor can be calculated from the intercept.

A differential isoconversional method devised by Freidman [25] has led to the commonly used equation:

$$\ln\left[\beta(\mathrm{d}\alpha/\mathrm{d}t)_{\alpha,\mathrm{i}}\right] = \mathrm{In}[f(\alpha) \times A] - E_a/RT_{\alpha,\mathrm{i}} \tag{4}$$

where $(\mathrm{d}\alpha/\mathrm{d}T)_{\alpha,\mathrm{i}}$ is the derivative of the α versus T curve at a fixed degree of conversion, α_i, and $T_{\alpha,\mathrm{i}}$ is the corresponding temperature at this point. At a fixed degree of conversion, $f(\alpha)$ will be a constant for a given set of experimental TG curves that are run under different heating rates and, as such, the $\ln[f(\alpha)\times A]$ term in eq. (4) will be a constant for the set of runs. Consequently, a plot of $\ln[\beta(\mathrm{d}\alpha/\mathrm{d}t)_{\alpha,\mathrm{i}}]$ versus $1/T_{\alpha,\mathrm{i}}$ will yield a straight line from which a value of the apparent activation energy can be obtained from the gradient.

2.3. Non-isothermal TGA data analysis algorithms

Algorithms were devised to facilitate a convenient method of analysis for a wide range of non-isothermal TG data. These computer-based algorithms are: (i) an algorithm for identifying suitable kinetic models for fitting the experimental data based on theoretical reference characteristics calculated by Dollimore et al. [16] for a given set of (T, α) input data and (ii) an iterative arithmetic algorithm that solves eq. (2) without invoking assumptions or approximations in order to extract the two Arrhenius parameters. The overall approach delivers the so-called "kinetic triplet" information and also enables one to assess whether the analysis has been appropriate in so far as the degradation occurred by a single mechanism over the temperature range.

2.3.1. *Algorithm to identify kinetic model*

This algorithm systematically calculates from the experimental (T, α) data the values of three characteristic parameters $(\mathrm{d}\alpha/\mathrm{d}T)_{\max}$, $\alpha_{\max}$ and ΔT, where $(\mathrm{d}\alpha/\mathrm{d}T)_{\max}$ is the maximum value of the derivative of α with respect to temperature, $\alpha_{\max}$ is the value of α at which the maximum derivative value occurs, and ΔT is the half-height width of the $\mathrm{d}\alpha/\mathrm{d}T$ versus T plot. The parameters are then compared with the corresponding ranges for these given in the reference data [16] for a number of different kinetic models, and a fit parameter, ρ, is calculated for each model. The fit parameter is such that $0 \le \rho \le 1$ and $\rho = 1$ is deemed to be a "perfect" fit. Details of the calculation of the fit parameter and the four possible cases under which it is calculated are given in the Supplementary Material.

2.3.2. *Iterative numerical TG analysis algorithm*

Taking the natural logarithm of both sides of eq. (2) and allowing for separate experimental measurements at different values of α_i as well as allowing for different values of the activation energy yields:

$$\delta_i(\alpha_i,\ E_{a,j}) = \ln[g(\alpha_i)] - \ln[p(x_i)] = AE_{a,j}/R\beta \tag{5}$$

where $\delta_i(\alpha_i, E_{a,j})$ represents a single value of a difference function that exists for a given value of α_i and its corresponding x_i value. The value of x_i at a given value of $E_{a,j}$ is calculated from $x_i = E_{a,j}/RT_i$ where T_i is the temperature corresponding to the particular α_i value. Details of the application of eq. (5) in an iterative process to identify the optimum value of E_a and the corresponding value of the Arrhenius A-factor associated with the experimental data are provided in the Supplementary Material.

3. Experimental section

Poly(lactic acid) (7001D Ingeo™; specific gravity 1.24; melt-flow index 6 g/10 min at 210 °C and 2.16 kg; melting temperature range 145–160 °C) was obtained from NatureWorks LLC, USA. Kenaf fibre (bast) was purchased from Ecofibre Industries, Australia.

To improve compatibility with the PLA matrix and remove any impurities, a preliminary surface treatment of the kenaf was performed by immersing it in 5% w/v NaOH (Merck Chemicals, Australia) for 2 h at room temperature. The fibres were then washed with and rinsed with distilled water. Acetic acid (Merck Chemicals, Australia) was used to adjust the pH to neutrality prior to a final rinse with distilled water. The fibres were filtered and dried overnight in an air circulating oven at 105 °C.

Prior to mixing, the PLA resin was dried in an oven at 60 °C. The PLA and treated kenaf fibres (40% w/w) were compounded using an internal mixer (Haake PolyLab OS, Germany) at 50 rpm and 155 °C for 8 min. The material was then collected from the mixer, cooled and preheated in a laboratory press (L0003, IDM Instruments Pty. Ltd., Australia) at 150 °C for 2 min. It was pressed at the same temperature for 3 min under a pressure of 50 kN before quench cooling to 30 °C under pressure to produce either films (0.3 mm thickness) or slabs (3 mm thickness) of material for analysis.

A Mettler Toledo TGA/DSC1 STARe system thermogravimetric analyzer was used to undertake the TG analyses. The PLA and composite samples were heated from 30 to 500 °C at a rate of 5 K min^{-1} and under a nitrogen atmosphere at a flow rate of 0.2 L min^{-1}.

4. Results and discussion

4.1. *Validation of the model identification and data fitting protocols*

In order to validate the previously described model identification and data fitting algorithms, a TG profile whose characteristics are known [18] was deconstructed and its (T, α) data analyzed as test data. The characteristics of these data are: contracting volume kinetic model, $E_a = 100$ kJ mol^{-1}, $A = 1.883 \times 10^{15}$ min^{-1} and heating rate $\beta = 1.000$ K min^{-1}.

Table 1 lists the values of ρ that were produced by the model identification algorithm along with the corresponding values of the linear regression coefficient, R^2, obtained from the analysis of plots of $g(\alpha)$ versus $p(x)$ that were also generated over the range of common kinetic models. Inspection of the values of ρ confirms that the test data conform to a contracting volume model ($\rho = 0.913$), as expected. However, it is also apparent that other models such as the Avrami-Erofeev A2 and A3 models ($\rho = 0.953$ in each case) may also be appropriate choices for fitting the data.

Table 1
Values of the model fitting parameter, ρ, and the corresponding linear regression coefficient, R^2, pertaining to the $g(\alpha)$ versus $p(x)$ plot obtained from the analysis of the contracting volume test data set under the various kinetic models.

Kinetic Model	$g(\alpha)$	ρ	R^2
Acceleratory			
Pn Power law	$\alpha^{1/n}$	0.69	note[1]
E1 Exponential law	$\ln(\alpha)$	0.55	0.6275[2]
Sigmoidal			
A2 Avrami-Erofeev	$[-\ln(1-\alpha)]^{1/2}$	0.95	0.9851
A3 Avrami-Erofeev	$[-\ln(1-\alpha)]^{1/3}$	0.95	0.9914
A4 Avrami-Erofeev	$[-\ln(1-\alpha)]^{1/4}$	0.69	0.9929
B1 Prout-Tompkins	$[-\ln(\alpha/(1-\alpha))] + C$	0.64	note[3]
Deceleratory			
Geometrical			
R2 Contracting area	$1-(1-\alpha)^{1/2}$	0.88	0.9950
R3 Contracting volume	$1-(1-\alpha)^{1/3}$	0.91	0.9996
Diffusion			
D1 One dimensional	α^2	0.79	0.8955
D2 Two dimensional	$(1-\alpha)\ln(1-\alpha) + \alpha$	0.79	0.9531
D3 Three dimensional	$[1-(1-\alpha)^{1/3}]^2$	0.66	0.9979
D4 Ginstling-Brounshtein	$1-(2/3)\alpha-(1-\alpha)^{2/3}$	0.73	0.9777
Reaction Order			
F1 First order	$-\ln(1-\alpha)$	0.87	0.9582
F2 Second order	$1/(1-\alpha)$	0.58	0.5440
F3 Third order	$1/(1-\alpha)^2$	0.16	0.0735

Notes.
1. Not fitted.
2. No convergence using fitting algorithm. Fitted using derivative method.
3. No convergence using fitting algorithm.

Fig. 1 shows a plot of the linear regression coefficient, R^2, versus ρ that was constructed from the data whose analyses are given in Table 1. Although some clustering around the higher values of the fit parameters is observed, the plot shows a reasonably good overall correlation between the two variables, suggesting that the proposed model identification algorithm can be used to distinguish suitable kinetic models for fitting the TG data.

The clustering of the data associated with the various models (Fig. 1) is a possible manifestation of the established fact that the same kinetic process can be described by different reaction models as well as by combinations of different apparent activation energy and Arrhenius A-factor values [14]. It has been suggested that almost any model can satisfactorily fit data if wide variations in the apparent activation energy and Arrhenius A-Factor are allowed [14]. Indeed, in the case of solid-state kinetics, this became apparent through the early work of Huttig et al. [26], and Bruzs [27], and had led to the iso-conversional method of evaluation proposed by Kujirali and Akahira [28].

The iterative numerical application of eq. (5) to the test data produces a convergence for the contracting volume kinetic model at an apparent activation energy value of $E_a = 100$ kJ mol^{-1} precisely (see also Supplementary Material), as expected from the prior knowledge of the input test data. The corresponding value of the A-factor produced by the algorithm is $A = 1.906 \times 10^{15}$ min^{-1} which is acceptably close to the expected value of $A = 1.883 \times 10^{15}$ min^{-1}. The appropriate fit of the test data to the expected contracting volume kinetic model is pre-empted by the value of ρ being close to unity, as indicated in Table 1. The fit of the model can be more authoritatively confirmed by plotting g(α) versus p(x), as shown in Fig. 2.

The linearity of the plot of g(α) versus p(x) in accordance with eq. (2) confirms that the model is appropriate and provides a high level of confidence in the value of the apparent activation energy that is obtained under the given model for the process (see Fig. 2). It also validates the assumption that the apparent activation energy remains constant over the temperature range of the experiment, suggesting that the mechanism does not change over the course of the applied temperature ramp. Furthermore, the value of the apparent activation energy obtained from the gradient of the plot (see eq. (2)) is 97.9 kJ mol^{-1} and is acceptably close to that obtained by the iterative arithmetic algorithm. A value for the apparent activation energy can also be determined by the differential method from an appropriate plot in accordance with eq. (3) where $f(\alpha) = 3(1-\alpha)^{2/3}$ in the case of a contracting volume model. Such a plot is shown in Fig. 3.

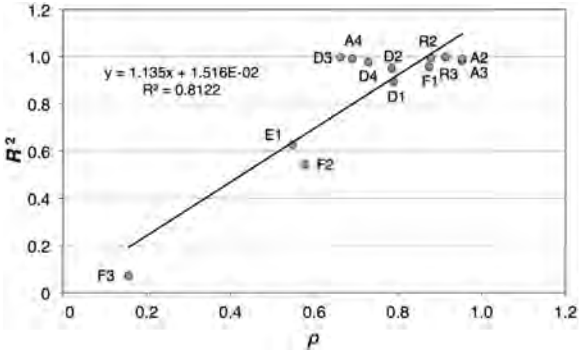

Fig. 1. Plot of ρ values produced by the model identification algorithm for the test data [18] versus corresponding values of the linear regression coefficient, R^2, obtained from the regression analysis of g(α) versus p(x) plots for the selection of common kinetic models.

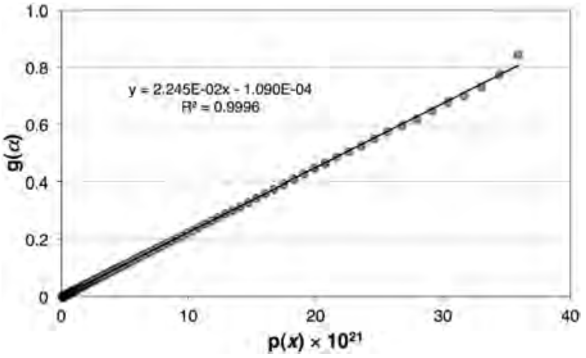

Fig. 2. Plot of g(α) versus p(x) for the test data [18] where $E_a = 100$ kJ mol^{-1} and g(α) = $1-(1-\alpha)^{1/3}$ (i.e. contracting volume model). The value of the apparent activation energy was that determined by the iterative numerical algorithm for solving eq. (2).

The gradient and intercept of the plot shown in Fig. 3 can be used to calculate values of the kinetic parameters: $E_a = 93.7$ kJ mol^{-1} and $A = 1.606 \times 10^{14}$ min^{-1}. Clearly, these values obtained *via* a differential method are significantly lower than the expected values and reflect the problem identified by Vyasovkin et al. [14] in that differentiation of “integral” type data, such as those data obtained from TGA experiments, tends to magnify the noise (as is clearly apparent at low levels of conversion). Therefore, it has been suggested that integral and differential methods are best suited for, respectively, analyzing integral (e.g. TGA) and differential (e.g. DSC, DTA) data [14].

4.2. Thermal degradation of poly(ethylene adipate)

The application of TGA to studying the thermal degradation of poly(ethylene adipate) is reported [21] in a paper

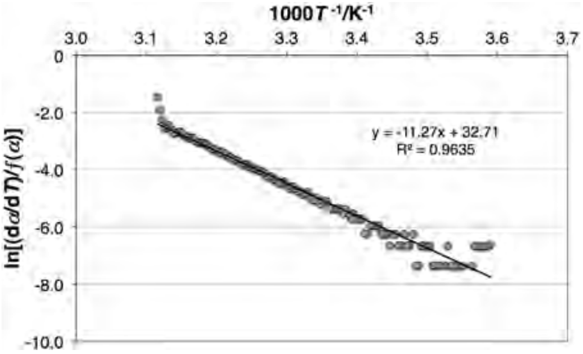

Fig. 3. Differential method plot of ln[(dα/dT)/$f(\alpha)$] versus 1/T in accordance with eq. (3) for the test data [18]. In this case $f(\alpha) = 3(1-\alpha)^{2/3}$ for a contracting volume model.

that compares a range of kinetic models along with an isoconversional method. The relevant data in this reference paper pertaining to four different experimental heating rates were deconstructed and processed (up to $\alpha = 0.8$ in all cases) as above in order to further assess the proposed algorithms.

Table 2 lists the values of the model fitting parameter, ρ, and the average of this parameter, ρ_{ave}, across the different experimental heating rates obtained from the analysis of the reference data [21]. The processed data indicate that the best kinetic fit may lie amongst the R3, D2, D3, D4 or F1 models, each of which has a ρ_{ave} value of *ca.* 0.9 or above. The numerical algorithm was used to fit the data at the different heating rates to each of the models listed in Table 2 and these results appear in Table 3.

Table 3 lists the values of the apparent activation energy, $E_{a,calc}$, and the decadic logarithm of the A-factor, $\log_{10}A_{calc}$ that were obtained using the iterative arithmetic algorithm to analyze the reference data [21]. Also listed in this table for comparison are $E_{a,ref}$ and $\log_{10}A_{ref}$ that are the overall values of the kinetic parameters reported in the literature [21].

The data in Table 3 exhibit wide variation in the kinetic parameters across the range of kinetic models, again illustrating that a given set of kinetic data may be fitted by a number of different kinetic triplets [14]. With the exception of the data obtained at a heating rate of 10 K min^{-1}, there is a consistent downward trend in the apparent activation energies as the heating rate is increased. This is consistent with the notion that for a given reaction model the kinetic parameters are expected to vary with heating rate [14], and highlights a possible limitation of non-isothermal gravimetric methods for unambiguously determining rate parameters within a given kinetic model.

Table 2

Values of the model fitting parameter, ρ, and the average of this parameter, ρ_{ave}, across the different experimental heating rates obtained from the analysis of reference data [21] for the thermal decomposition of poly(ethylene adipate).

Kinetic Model	Heating rate, β/K min^{-1}				ρ_{ave}
	5	10	15	20	
Acceleratory					
Pn Power law	0.01	0.07	0	0	0.02 ± 0.02
E1 Exponential law	0.52	0.52	0.52	0.52	0.52 ± 0.01
Sigmoidal					
A2 Avrami-Erofeev	0.78	0.80	0.74	0.78	0.77 ± 0.02
A3 Avrami-Erofeev	0.74	0.77	0.70	0.74	0.74 ± 0.02
A4 Avrami-Erofeev	0	0	0	0	0
B1 Prout-Tompkins	0	0.03	0	0	0.01 ± 0.01
Deceleratory					
Geometrical					
R2 Contracting area	0.92	0.94	0.76	0.85	0.87 ± 0.06
R3 Contracting volume	0.91	0.92	0.86	0.91	0.90 ± 0.02
Diffusion					
D1 One dimensional	0.68	0.72	0.54	0.61	0.64 ± 0.06
D2 Two dimensional	0.97	0.97	0.91	0.94	0.95 ± 0.02
D3 Three dimensional	0.97	0.98	0.96	0.99	0.98 ± 0.01
D4 Ginstling-Brounshtein	0.97	0.95	0.93	0.96	0.95 ± 0.01
Reaction Order					
F1 First order	0.94	0.94	0.98	0.98	0.96 ± 0.02
F2 Second order	0.82	0.83	0.92	0.87	0.86 ± 0.04
F3 Third order	0.57	0.56	0.71	0.64	0.62 ± 0.06

The TGA data for the decomposition of poly(ethylene adipate) were analyzed using a model-free approach using the isoconversional method proposed by Friedman [25] in accordance with eq. (4). The relevant plot of the processed data appears in Fig. 4.

The Friedman method of analysis of the data produces apparent activation energy values of $E_a = 156$ kJ mol^{-1} ($\alpha = 0.2$) and $E_a = 146$ kJ mol^{-1} ($\alpha = 0.8$). The magnitudes of these values are consistent with those reported in the original source but calculated using a different method [21]. The decrease in the apparent activation energy with increased extent of conversion suggests the assumption that the activation energy remains constant throughout the process, which is inherent in the data processing algorithm, is not strictly valid. Nonetheless, the variation of *ca.* 10 kJ mol^{-1} is considered to be small and inconsequential. For systems where this assumption is not valid, a correction procedure similar to the one described by Vyazovkin [24], for handling integral isoconversional data would need to be invoked.

The apparent activation energy range determined in the Friedman analysis is closest to those values of this parameter that are produced by the iterative arithmetic algorithm for the R2, R3 and F1 models (see Table 3). The R3 and F1 models were identified by the model identification algorithm as being appropriate candidates for data fitting with respective average fit parameters of $\rho_{ave} = 0.90 \pm 0.02$ and $\rho_{ave} = 0.96 \pm 0.02$ (see Table 2). The quality of this fit is also confirmed by the respective linear regression coefficients associated with the g(α) versus p(x) plots for these systems determined at a heating rate of 5 K min^{-1}, which are $R^2 = 0.9913$ and $R^2 = 0.9753$, respectively. Given the highly non-linear nature of the functions g(α) and p(x), it is anticipated that the extent to which a plot of g(α) versus p(x) conforms to linearity will be a sensitive indicator of the goodness of fit of the model and, consequently, the validity of the assumption that the apparent activation energy remains constant over the temperature range of the experiment.

Considering only those models where the fit parameter was 0.9 or above (i.e. the R3, D2, D3, D4 or F1), the congruency between the apparent activation energies delivered by the iterative arithmetic algorithm, $E_{a,calc}$ and the corresponding reference values [21], $E_{a,ref}$ can be tested by plotting these quantities against each other (Fig. 5).

Plots similar to that shown in Fig. 5 can be constructed for the data obtained at the other heating rates and all show a similar trend. The regression line of best fit through the data and that passes through the origin shows a reasonably high level of correspondence between the variables in as far as its gradient is close to unity and the linear regression coefficient is also reasonably high. Thus, the values of the apparent activation energy delivered by the iterative arithmetic algorithm are reasonably consistent with those values reported in the literature obtained at a heating rate of 5 K min^{-1}. The differences between the $E_{a,calc}$ values for the R3 and F1 models and the corresponding values delivered by the model-free analysis [25] may be due in part to the assumption inherent in the arithmetic algorithm that the apparent activation energy remains constant over the temperature range of the experiment.

Table 3

Values of the apparent activation energy, $E_{a,calc}$, and the decadic logarithm of the A-factor, $\log_{10}A_{calc}$ that were obtained from reference data [21] for the thermal decomposition of poly(ethylene adipate) at different heating rates. The overall values reported for the kinetic parameters ($E_{a,ref}$, $\log_{10}A_{ref}$) [21] are listed for comparison.

Kinetic Model	$E_{a,calc}$/kJ mol^{-1}				$E_{a,ref}$/kJ mol^{-1}	$\log_{10}A_{calc}$ [1]				$\log_{10}A_{ref}$ [1]
	Heating rate β/K min^{-1}					Heating rate β/K min^{-1}				
	5	10	15	20		5	10	15	20	
Sigmoidal										
A2 Avrami-Erofeev	64	51	60	57	123.0	2.47	1.50	2.38	2.22	7.68
A3 Avrami-Erofeev	40	31	37	35	114.7	0.34	−0.27	0.40	0.33	7.00
B1 Prout-Tompkins	nc[2]	nc	nc	nc	118.1	nc	nc	nc	nc	8.35
Deceleratory										
Geometrical										
R2 Contracting area	131	100	125	115	153.1	7.83	5.25	7.41	6.62	9.74
R3 Contracting volume	133	99	126	117	159.6	7.85	5.01	7.34	6.65	10.13
Diffusion										
D1 One dimensional	261	209	250	228	220.6	18.79	14.29	17.59	15.73	15.23
D2 Two dimensional	269	211	257	237	246.1	19.26	14.23	17.95	16.28	17.14
D3 Three dimensional	275	208	263	243	280.9	19.19	13.41	17.89	16.23	19.46
D4 Ginstling-Brounshtein	270	203	259	236	257.5	18.72	12.94	17.51	15.58	17.46
Reaction Order										
F1 First order	137	110	128	123	174.7	8.71	6.46	8.00	7.66	11.93
F2 Second order	8	5	5	4	237.1	−2.51	−2.61	−2.44	−2.46	17.30

Notes.
1. Units of A are s^{-1}.
2. Data were not convergent (nc) using the analysis algorithm.

4.3. Thermal degradation of poly(lactic acid)/kenaf composites

To further test the applicability of the proposed algorithms, samples of poly(lactic acid) (PLA) that were filled with 40% w/w of the natural fibre kenaf were degraded in nitrogen over the temperature range of 200 to 500 °C. These materials are of current interest as a naturally derived substrate for formulations of novel packaging materials that contain natural antimicrobial agents [29]. Fig. 6 shows plots of α versus temperature for both the neat PLA and the filled sample. The plot for the composite clearly shows two steps: the first being due to the thermal degradation of PLA and the second due to the decomposition of the kenaf that ultimately leads to a char residue.

The model identification algorithm identified the contracting volume model for degradation (R3) as the most appropriate basis on which to analyze the TG data pertaining to the degradation of the PLA. The kinetic analyses of these data in accordance with the R3 model suggest that the apparent activation energy for the degradation of PLA is *ca.* 190 kJ mol^{-1}, whereas in the presence of the kenaf filler this value is significantly lowered to *ca.* 150 kJ mol^{-1}. This suggests the addition of the kenaf to the PLA matrix destabilizes the polymer.

Furthermore, the data in Fig. 6 corresponding to the degradation of the TK fibres (i.e. the data from *ca.* 350 to 460 °C) were analyzed separately and found to conform closely to a first-order kinetic process (i.e. $g(\alpha) = -\ln(1-\alpha)$) and were fitted accordingly. The analysis produced a value of $E_a = 314$ kJ mol^{-1} for this process which is very high compared to the value of *ca.* 169 to 170 kJ mol^{-1} obtained by Yao et al. [30]. This difference may be due to the alkali

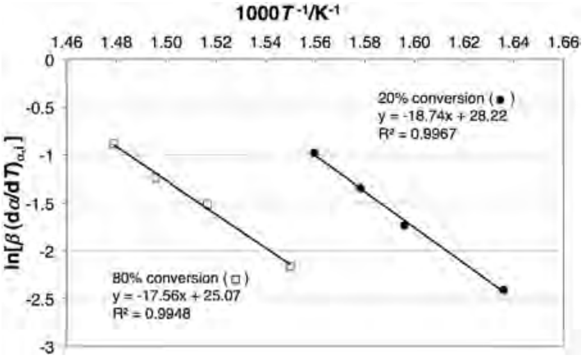

Fig. 4. Differential isoconversional method plot of $\ln[\beta(d\alpha/dT)_{\alpha,i}]$ versus $1/T_{\alpha,i}$ for the thermal decomposition of poly(ethylene adipate) in accordance with the method devised by Freidman [25].

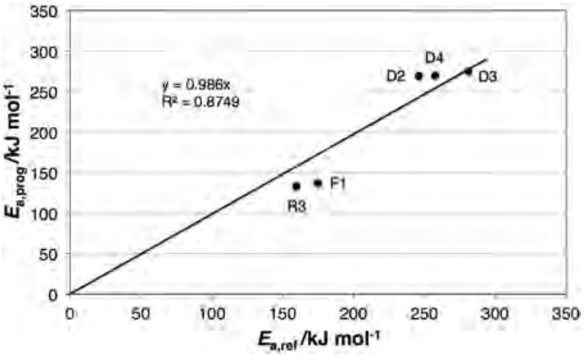

Fig. 5. Plot of $E_{a,calc}$, the apparent activation energy obtained using the iterative arithmetic algorithm for the R3, D2, D3, D4 and F1 models versus $E_{a,ref}$, the corresponding overall apparent activation energy reference value reported for the thermal decomposition of poly(ethylene adipate) [21] at a heating rate of 5 K min^{-1}.

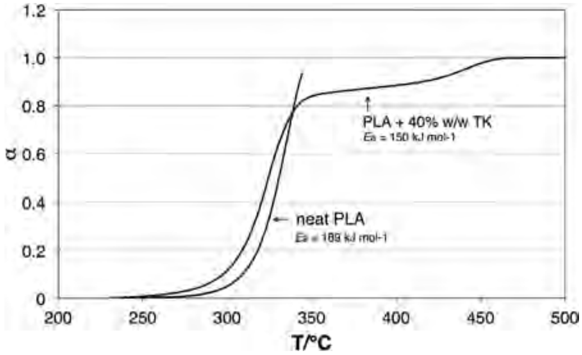

Fig. 6. Plot of α versus T for: (i) neat PLA and (ii) PLA filled with 40% w/w kenaf. The samples were degraded in nitrogen over the temperature range of 200 to 500 °C at a heating rate of 5 K min^{-1}.

pre-treatment of the fibres used in the present study which chemically alters much of the lignin, hemicellulose and other material on their surface that may, in turn, destabilize and/or catalyze the decomposition of the kenaf [31,32]. Discrepancy may also arise as a result of the data selected for analysis being convoluted in the early part of the trace with those data associated with the final part of the PLA decomposition. It is anticipated, however, that failure to deconvolute these data prior to analysis would not be solely responsible for the large difference between the two values.

5. Conclusions

Iterative arithmetic techniques can, where appropriate, provide a convenient means by which integral type thermochemical data can be processed for the routine assessment of polymer degradation kinetics. The model identification algorithm exhibits significant promise as a method by which applicable kinetic models can be identified. The iterative arithmetic algorithm for the calculation of the kinetic parameters provides a way of solving the Arrhenius integral and associated equations without the necessity of making mathematical approximations and assumptions in the processing of the TG experimental data. Nonetheless, it is expected that such analyses will only have validity in the case of simple systems where a single degradation mechanism prevails across the range of temperatures and heating rates used in the experiment. The linearity of the g(α) versus p(x) plot serves as a check on the validity of any assumptions that may have been made.

The decomposition of poly(ethylene adipate) may be described by a kinetic triplet involving a first-order (F1) mechanism with an apparent activation energy of 137 kJ mol^{-1} and $\log_{10}A = 8.71$. Neat PLA and PLA composites each decompose in accordance with the contracting volume (R3) model where the addition of kenaf filler to the PLA destabilizes the latter, lowering its apparent activation energy from *ca.* 190 kJ mol^{-1} to *ca.* 150 kJ mol^{-1}.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.polymertesting.2015.03.002.

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Chapter 6 – Thymol Interactions in PLA Composites

Interaction and Quantification of Thymol in Active PLA-Based Materials Containing Natural Fibers

Overview

Chapter 6 examines the quantification/retention of thymol in PLA and PLA/kenaf films after thermal processing with a discussion on the interactions of thymol between the PLA matrix and the kenaf fibre filler. The algorithms of the thermogravimetric (TG) assessment of the PLA and PLA/kenaf composites containing thymol degradation under non-isothermal was used as a tool to investigate the interactions between the thymol, the kenaf fibres and the PLA matrix as reported in this chapter. The potential applications of these systems as AM food-packaging materials are also considered.

The paper entitled "Interaction and Quantification of Thymol in Active PLA-Based Materials Containing Natural Fibers" by Tawakkal I. S. M. A., Cran M. J. and Bigger S. W. was published in the *Journal of Applied Polymer Science,* **132**, 42160 (1-11), 2016.

A part of the research work was selected as the Cover Image in Journal of Applied Polymer Science, Special Issues: Bio-Based Packaging, Volume 133, Issues1-2, 2016.

PART B:

DECLARATION OF CO-AUTHORSHIP AND CO-CONTRIBUTION: PAPERS INCORPORATED IN THESIS BY PUBLICATION

This declaration is to be completed for each conjointly authored publication and placed at the beginning of the thesis chapter in which the publication appears.

Declaration by [candidate name]: **Signature:** [redacted] **Date:** 05.03.2015

Intan S. M. A. Tawakkal

Paper Title:

Interaction and Quantification of Thymol in Active PLA-Based Materials Containing Natural Fibers

In the case of the above publication, the following authors contributed to the work as follows:

Name	Contribution %	Nature of Contribution
Intan S. M. A. Tawakkal	70	Designed the experiment Performed the sample analyses Prepared major part of the manuscript
Marlene J. Cran	20	Contribution to writing of paper and journal submission
Stephen W. Bigger	10	Contribution to writing of paper

DECLARATION BY CO-AUTHORS

The undersigned certify that:

1. They meet criteria for authorship in that they have participated in the conception, execution or interpretation of at least that part of the publication in their field of expertise;
2. They take public responsibility for their part of the publication, except for the responsible author who accepts overall responsibility for the publication;
3. There are no other authors of the publication according to these criteria;
4. Potential conflicts of interest have been disclosed to **a)** granting bodies, **b)** the editor or publisher of journals or other publications, and **c)** the head of the responsible academic unit; and
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Chapter 7 – Release of Thymol from PLA Films

Release of Thymol from PLA-Based Antimicrobial Films Containing Kenaf Fibres as Natural Filler

Overview

In this chapter, the migration of thymol from the PLA and PLA/kenaf films into food simulants is examined. The release rates of thymol into simulants at different temperatures were determined by using first-order kinetics, diffusion modelling and Fick's law modelling with a comparison of the various models. The final material appearance after the release of thymol into simulants is also reported and discussed.

The paper entitled "Release of Thymol from PLA-Based Antimicrobial Films Containing Kenaf Fibres as Natural Filler" by Tawakkal I. S. M. A., Cran M. J. and Bigger S. W. was published in *LWT-Food Science and Technology*, **66**, 629-637, 2016.

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Intan S. M. A. Tawakkal	70	Designed the experiment Performed the sample analyses Prepared major part of the manuscript
Marlene J. Cran	20	Contribution to writing of paper and journal submission
Stephen W. Bigger	10	Contribution to writing of paper

DECLARATION BY CO-AUTHORS

The undersigned certify that:

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Release of thymol from poly(lactic acid)-based antimicrobial films containing kenaf fibres as natural filler

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ABSTRACT

The migration of thymol, a natural antimicrobial (AM) substance, from poly(lactic acid) (PLA) films containing 300 g/kg kenaf fibres into food simulants is reported. Neat PLA and PLA/kenaf films containing 100 g/kg thymol were prepared *via* melt blending and heat pressing and were placed in contact with 150 mL/L and 950 mL/L ethanol/water mixtures at different temperatures. First-order kinetics, diffusion modelling and Fick's law modelling were used to describe the release. The release rate of thymol into 950 mL/L ethanol/water at different temperatures displays Fickian behavior with diffusion coefficient values between 1 and 100 × 10^{-11} m^2 s^{-1} with close to 100% of thymol being released. The release rate of thymol is temperature dependent and is affected by the percentage of ethanol in the simulant. In the case of neat PLA and PLA/kenaf films, a faster release occurred in 950 mL/L ethanol/water than in 150 mL/L ethanol/water with the composite film exhibiting a higher diffusion coefficient in each case.

1. Introduction

Controlled release systems in food packaging have experienced considerable growth recently due to developments in active packaging concepts such as the integration of antioxidant (AOX) and/or antimicrobial (AM) substances into packages in order to improve the quality and safety of food products. In these systems, low molecular mass compounds and/or substances are released from the package in a slow and controlled manner to maintain an adequate concentration of the substance in the packed food for a certain period of time. The release of substances that involve migration is the result of diffusion, dissolution and equilibrium processes (Crank, 1979). There are various factors that influence the migration of a substance from the packaging material including the film fabrication method, the volatility and polarity of the substance, the chemical interaction between the substance and polymer chains, hydrophobicity and hydrophilicity of the polymer as well as food properties and composition (Suppakul, Miltz, Sonneveld, & Bigger, 2003).

Poly(lactic acid) (PLA) is a polyester synthesized from the renewable, bio-derived, monomer lactic acid and can be used as AM films and/or membranes for a different range of applications (Auras, Harte, & Selke, 2004; Karami, Rezaeian, Zahedi, & Abdollahi, 2013). This GRAS (Generally Recognized As Safe) grade polymer can be used in contact with food and fabricated *via* conventional processing procedures (Jamshidian, Tehrany, Imran, Jacquot, & Desobry, 2010). The use of fillers in combination with PLA has been widely studied with aims to improve physicomechanical properties, reduce production costs and enhance biodegradability. Naturally derived additives or fillers such as starch and cellulose can be combined with PLA and other active agents such as AM and AOX compounds. For example, Hwang et al. (2013) studied the migration of α-tocopherol and resveratrol from poly(L-lactic acid) (PLLA)/starch blend films into ethanol and found that the neat PLLA containing α-tocopherol had a lower release rate than the PLLA/starch blend films at 43 °C in 100% ethanol simulant with diffusivity coefficients of 89 × 10^{-11} and 282 × 10^{-11} cm^2 s^{-1} respectively. A similar trend was observed for resveratrol, a non-volatile AOX compound with diffusion rate of 25 × 10^{-11} cm^2 s^{-1} in neat PLLA and 40 × 10^{-11} cm^2 s^{-1} in the PLA/starch films. Fortunati et al. (2012) prepared PLA AM films with 50 g/kg microcrystalline cellulose (MCC) and 10 g/kg silver nanoparticles by extrusion and injection molding techniques. The PLA films with silver nanoparticles and MCC had greater AM activity against *Escherichia coli* due to the presence of MCC; however, it had less AM activity than PLA films with silver nanoparticles. Although there are examples of

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the controlled release of AM and AOX substances from PLA materials (Busolo & Lagaron, 2013; Fernandez, Soriano, Hernandez-Munoz, & Gavara, 2010; Hwang et al., 2013; Iniguez-Franco et al., 2012; Llana-Ruiz-Cabello et al., 2015), few reports have combined PLA with natural fibres in order to control the release of active substances from composite films.

Antimicrobial packaging systems can be categorised as either migratory or non-migratory systems. In the former, AM substances migrate from the packaging material into the headspace of the package and onto the food surface, whereas in the latter, AM substances are immobilised onto the packaging material which is placed in direct contact with the foodstuff to facilitate its activity (Han, 2003). The integration of PLA with AM substances has been investigated by a number of researchers (Del Nobile et al., 2009; Jin, 2010; Qin et al., 2015; Rhim, Hong, & Ha, 2009). Of the reported studies, many have investigated the inhibition of targeted microorganisms with little attention having been devoted to studying the release rate from the active systems. This might be due to the preponderance of non-volatile and/or immobilised AM substances (e.g. nisin, chitosan, lysozyme and peptide) incorporated into PLA films rather than volatile AM substances (Green, Fulghum, & Nordhaus, 2011; Rhim, 2013; Tawakkal, Cran, Miltz, & Bigger, 2014b). Several AM substances have been incorporated directly into polymers including a range of volatile plant extracts such as basil, thymol, linalool, methyl cinnamate and cavacrol (Cran, Rupika, Sonneveld, Miltz, & Bigger, 2010; Del Nobile, Conte, Incoronato, & Panza, 2008; Fernández-Pan, Maté, Gardrat, & Coma, 2015; Rubilar et al., 2013; Suppakul, 2004; Suppakul, Sonneveld, Bigger, & Miltz, 2011; Tawakkal, Cran, & Bigger, 2015).

Thymol, an essential oil extract that has GRAS status, can be used in contact with food products and like other volatile AM substances, the migration of this substance into real food products is complex. In some studies, active AM films containing thymol were evaluated *in vitro* and *in vivo* against a wide spectrum of microorganisms such as bacteria, mould and yeast (Kuorwel, Cran, Sonneveld, Miltz, & Bigger, 2011; Wu et al., 2014). In a recent study by Petchwattana and Naknaen (2015), extruded films of poly(butylene succinate) (PBS) containing thymol demonstrated AM activity against *E. coli* and *Staphylococcus*. Moreover, the release rate of thymol from the PBS films into 950 mL/L ethanol/water was found to be 5.9×10^{-14} m^2 s^{-1}. Ramos, Beltrán, Peltzer, Valente, and Garrigós (2014a) reported that the migration of thymol from polypropylene (PP) films into 950 mL/L ethanol at 40 °C conformed to Fick's law with a diffusion coefficient of 1.0×10^{-14} m^2 s^{-1}. In general, an AM substance can be released into a food simulant by swelling-controlled release with three main steps involved: (i) the absorption of fluid (penetrant) from the food simulant which leads to the swelling effect of the polymer, (ii) the active substance molecule being dissolved or dispersed in the polymeric matrix and (iii) the active substance migrating to the food simulant (Del Nobile & Conte, 2013).

The quantification of volatile AM substances in active films is an important consideration given that high pressure, shear forces and temperatures are required for film processing. According to Raouche, Mauricio-Iglesias, Peyron, Guillard, and Gontard (2011), a higher temperature of *ca*. 160–190 °C was needed to process PLA using extrusion which may easily degrade and evaporate the volatile AM substance during thermal processing. The retention of volatile additives such as thymol in PLA film was found to be approximately 70–80% after thermal fabrication (Tawakkal et al., 2015). Such retention percentages were significantly higher than those found in polyolefin film systems containing similar volatile additives. For example, Ramos, Jiménez, Peltzer, and Garrigós (2012) reported much lower retention of thymol and carvacrol (*ca*. 25–45% respectively) in PP formulations. The marked reduction in the retention of these additives in polyolefin films upon thermal processing may result from the reduced compatibility between the polymer matrix and the natural additives as well as the processing parameters such as temperature, time and screw rotation (Del Nobile et al., 2009).

There are clearly many examples of the release of a wide range of AM substances from homopolymers such as PLA. However, little attention has been devoted to evaluating the release of AM agents from ternary composite systems, particularly taking into account the possible swelling of polymeric materials immersed in food simulants as well as the effects of natural fillers on the release rate. Moreover, the release of volatile AM substances from polymeric materials also has been restricted to mainly hydrophobic and moderate hydrophilic polymeric matrices (Buonocore, Del Nobile, Panizza, Corbo, & Nicolais, 2003; Herath, 2009). The aim of the current work was therefore to investigate the release of a naturally derived AM agent from a novel ternary system comprised of PLA, kenaf fibers and thymol. In particular, the release of thymol from PLA and PLA/kenaf composite and its diffusion kinetics were studied.

2. Experimental section

2.1. Materials

Poly(lactic acid) (7001D Ingeo™; specific gravity 1.24; melting temperature 152.5 °C (Tawakkal, Cran, & Bigger, 2014a)) was obtained from NatureWorks LLC, USA. Mechanically separated kenaf fibre (bast) was purchased from Ecofibre Industries, Australia. Thymol (T0501, purity of 99.5%) was purchased from Sigma Aldrich Pty. Ltd., Australia. Sodium hydroxide and acetic acid were purchased from Merck Chemicals, Australia. Un-denatured ethanol was purchased from Chem-Supply Pty Ltd., Australia. Isooctane (2,2,4-trimethylpentane, 36006) was purchased from Sigma Aldrich, Australia.

2.2. Production of PLA/kenaf/thymol films

Kenaf fibres were soaked in 0.05 g/mL sodium hydroxide for 2 h at room temperature. The fibers were then filtered and washed with distilled water prior to acid treatment to affect neutralization by adding a few drops of acetic acid. The fibres were then filtered, washed and rinsed with distilled water to remove the acetic acid, the latter being confirmed using a pH meter (inoLab® pH7110, WTW GmbH, Germany). Finally, the fibres were dried overnight in an oven at 105 °C. Prior to mixing, PLA resin and kenaf fibres were further dried in an oven at 60 °C overnight before mixing with thymol at 100 g/kg concentration.

The film samples were prepared firstly by melt-blending the components in an internal mixer (Haake PolyLab OS, Germany) at 155 °C for 8 min and 50 rpm followed by heat pressing, according to a method previously reported by Tawakkal, Cran, and Bigger (2014a). The PLA was added to the mixer first and the kenaf and thymol were introduced once the polymer was molten in order to avoid unnecessary loss of thymol. In the current study, a 300 g/kg loading of kenaf fibres was used to produce the PLA/kenaf composite that has moderate flexibility as well as high strength and stiffness compared with unfilled PLA which is more suitable for the production of rigid packaging applications (Tawakkal, Cran, & Bigger, 2014a). In a second step, a laboratory press (L0003, IDM Instrument Pty. Ltd., Australia) was used to prepare films. The samples were preheated at 150 °C for 3 min without applying pressure until the material melted, and then pressed at the same temperature for 2 min under a force of 20 kN before quench cooling to 30 °C under pressure. The average thicknesses of the pressed neat PLA and PLA/kenaf films incorporated with thymol were 0.19 ± 0.03 and 0.25 ± 0.05 mm respectively. The film thickness was measured using a hand-held micrometer (Hahn & Kolb, Stuttgart, Germany).

2.3. Quantification of thymol in PLA and PLA/Kenaf films

One gram of film sample was cut into pieces (0.25 cm^2) and immersed in a round bottom flask containing 150 mL of isooctane for solid–liquid extraction. Isooctane was used as it promotes the swelling of the polymer and a typical reflux extraction was performed at 100 °C for 4–5 h to extract thymol from the films. The quantification was achieved with a gas chromatography (GC) instrument (Varian 8200C_x) equipped with a fused silica capillary column (DB5; 30 m × 0.25 mm i.d.; thickness 0.25 μm; J & W Scientific, USA). The conditions applied in the GC instrument were as follows: injected volume: 1.0 μL, initial column temperature: 80 °C; heating rate: 5 °C min^{-1} up to 120 °C, held at this temperature for an additional 5 min; injector temperature: 250 °C; FID detector temperature: 300 °C; flow rate: 2 mL min^{-1}; splitting; carrier gas: nitrogen. Standard solutions of thymol in isooctane at concentrations from 0.05 to 1.0 mg mL^{-1} were prepared and used to produce a calibration curve. The experiments were performed in triplicate. The retention of thymol in the neat PLA and PLA/kenaf films following thermal processing was 73% and 62% respectively as reported by Tawakkal et al. (2015). The loss of thymol observed in the present study for composite films seems to be at an acceptable level compared to petroleum-based (polyolefin) films containing volatile additives.

2.4. Migration of thymol into food simulants

The release of thymol from neat PLA and PLA/kenaf films into aqueous food simulants was studied at different temperatures. The release was examined *via* a total immersion migration test (EC., 1997) using 950 and 150 mL/L ethanol/water. Ethanol is commonly used as food simulant to investigate the migration of AM substances from the PLA matrix. The 950 and 150 mL/L ethanol/water simulants are fatty and aqueous food simulants respectively. The sorption of ethanol by the PLA matrix may lead to the creation of voids and/or swelling of the matrix where it can penetrate the PLA chains and promote the migration of the active substance (Mascheroni, Guillard, Nalin, Mora, & Piergiovanni, 2010). In the case of the 950 mL/L ethanol/water simulant the experiments were performed at 30, 40, 50 and 60 °C and for the 150 mL/L ethanol/water simulant experiments were performed at 60, 65, 75 and 83 °C. In the latter experiments, the test temperatures were higher than recommended in the standard methods (EC., 1997) in order to accelerate the migration of thymol. Studying the release rates of thymol from the neat PLA and PLA/kenaf films by using fatty and aqueous food simulants is important in order to assess the *in vitro* and *in vivo* AM activity of these systems against targeted microorganisms.

Samples of film weighing *ca.* 0.5–0.6 g were immersed in 100 mL of simulant in a three-neck round bottom flask with the ratio of simulant volume per area film being *ca.* 2.7–4.7 mL cm^{-2}. The flask was immersed in an oil bath that was placed on a magnetic stirrer heating plate and the simulant was gently agitated using magnetic stirring at 60 rpm. The flask was connected to a condenser and the stirring speed and temperature were fixed and monitored throughout the experiment. The amount of AM agent released from the films was monitored until equilibrium was attained. A 0.2 mL sample of the simulant solution was collected periodically during the experiment and 1 μL aliquots were injected into the GC. The quantification of thymol in all simulants was performed using the same method and calibration curve described in the previous section. The thymol quantification was performed in triplicate.

2.5. Data analysis

The migration of thymol from the PLA and PLA/kenaf composite films was analysed using three data analysis treatments: (i) overall kinetics, (ii) diffusion models in accordance with Cran et al. (2010) and Kuorwel, Cran, Sonneveld, Miltz, and Bigger (2013) and (iii) Fick's diffusion law model.

2.5.1. Overall kinetics analysis

By considering the overall diffusion process to be a single process that obeys first-order kinetics, equations describing the migration of an additive from a polymeric film into simulant with time have been described by Miltz (1987) and Crank (1979). The release of the AM agent into the simulant was initially analysed for its fit to a first order kinetics model. In the case of a first-order system, Equation (1) applies:

$$\ln\left(1-\frac{m_t}{m_\infty}\right)=-k_1t \tag{1}$$

where m_t is the mass of additive released from the film at time t, m_∞ is the amount of additive released from the film at equilibrium ($t=\infty$) and k_1 is the first-order rate constant. From Equation (1), a plot of $\ln(1-m_t/m_\infty)$ *versus* time should be a straight line with a slope of -k_1. The apparent first-order rate constants were calculated using Equation (1) and the initial release rates, v_0, of the AM agent were calculated using Equation (2) (Kuorwel et al., 2013):

$$v_0=m_\infty k_1 \tag{2}$$

2.5.2. Diffusion model

In the diffusion model, the release of the AM agent from the film into the simulant is considered in two stages, namely the short-term and the long-term (Crank, 1979; Miltz, 1987). This diffusion model is based on a geometry whereby the release is considered to occur from both sides of the film. The diffusion data were analysed using Equation (3) for short-term migration and the corresponding rate constants were calculated using Equation (4) for long-term migration.

Short-term migration is defined as the time for which $m_t/m_\infty <$ 0.6:

$$\frac{m_t}{m_\infty}=4\left(\frac{Dt}{\pi l^2}\right)^{\frac{1}{2}} \tag{3}$$

where D is the diffusion coefficient and l is the thickness of the film. A plot of m_t/m_∞ *versus* $t^{½}$ should yield a straight line from which the diffusion coefficient can be obtained.

For long-term migration $m_t/m_\infty > 0.6$ and Equation (4) applies:

$$\frac{m_t}{m_\infty}=1-\left(\frac{8}{\pi^2}\right)exp\left(-\frac{\pi^2Dt}{l^2}\right) \tag{4}$$

2.5.3. Fick's law model

The diffusion coefficient of the AM agent can be determined from its release *versus* time data, by fitting these data to Fick's second law. Equation (5) can be derived from Fick's second law in the case of one dimensional diffusion from a limited volume of film that is in contact with an infinite volume of solution (Crank, 1979).

$$\frac{m_t}{m_\infty} = 1 - \frac{8}{\pi^2}\sum_{m=0}^{\infty}\frac{1}{(2m+1)^2}exp\left[\frac{-D(2m+1)^2\pi^2 t}{l^2}\right] \qquad (5)$$

2.5.4. Diffusion activation energy

The effect of temperature on the release rate of the AM agent was modeled in accordance with the Arrhenius equation (Suppakul 2004):

$$D = D_0 exp\left(\frac{E_a}{RT}\right) \qquad (6)$$

where D is the diffusion coefficient, D_o is the pre-exponential factor, E_a is the activation energy for the diffusion process, R is the ideal gas constant, and T is the absolute temperature.

3. Results and discussion

3.1. Release of thymol into simulants

Fig. 1(a) and (b) show plots of the mass fraction m_t/m_∞ *versus* time, t and the overall kinetic analysis for the release of thymol from neat PLA and PLA/kenaf films into 950 mL/L ethanol/water at the four temperatures studied. The thymol release reached equilibrium within *ca.* 9 h at the lowest temperature of 30 °C (Fig. 1(a)). A similar observation was found for PLA containing resveratrol where the system achieved equilibrium after *ca.* 14 h using the same food simulant at 33 °C (Soto-Valdez, Auras, & Peralta, 2011). In comparison, the times required to achieve the equilibrium concentration of thymol in different polymeric systems with the same food simulant at 40 °C were found to be 50 h for PBS/thymol films and 150 h for PP/thymol films (Ploypetchara, Suppakul, Atong, & Pechyen, 2014; Ramos, Beltrán, Peltzer, Valente, et al., 2014a) and 2 h for starch/thymol films in isooctane at 35 °C (Kuorwel et al., 2013). In the current work, it was found that increasing the temperature to say 60 °C, increases the release rate of thymol and equilibrium was attained within 1.4 h. At the end of these experiments, *ca.* 87–100% and 82–95% of the thymol was released from the neat PLA and PLA/kenaf films respectively into 950 mL/L ethanol/water over the temperature range of 30–60 °C.

The initial release rates and the overall rate constants for the release of thymol that were calculated from the data in Fig. 1(b) are presented in Table 1. For both neat PLA and PLA/kenaf films, the initial release rates and the overall rate constants for thymol release into 950 mL/L ethanol/water consistently increased with an increase in temperature from 30 to 60 °C. This is consistent with the findings of Kuorwel et al. (2013) where an increase in temperature was found to have a significant effect on the migration of the additive from the film. From the results in Table 1 a similar observation can also be made in the case of the 150 mL/L ethanol/water simulant over the range of the four temperatures studied. However, in the latter case the rates are significantly lower compared with the 950 mL/L ethanol/water case. The experimental results were further analyzed by using the diffusion model in which the short-term and the long-term migration periods were considered.

Fig. 2(a) and (b) show the respective plots of m_t/m_∞ *versus* $t^{1/2}$ for the short-term release of thymol and $\ln(1 - m_t/m_\infty)$ *versus* t for the long-term release of thymol from the neat PLA and PLA/kenaf films into 950 mL/L ethanol/water at 30 °C. A similar behavior was observed at each of the temperatures that were studied and the linearity of the plots suggests that the data conform well to the diffusion model given in Equation (3) for short-term release. Values of the apparent diffusion coefficient at each of the various temperatures were determined from the gradients of these plots and are also presented in Table 1. For the long-term release, the linearity of the $\ln(1 - m_t/m_\infty)$ *versus* t plots also confirms the data reliably fit the model presented in Equation (4). Indeed, all results exhibited good linear correlation with correlation coefficients (r^2 values)

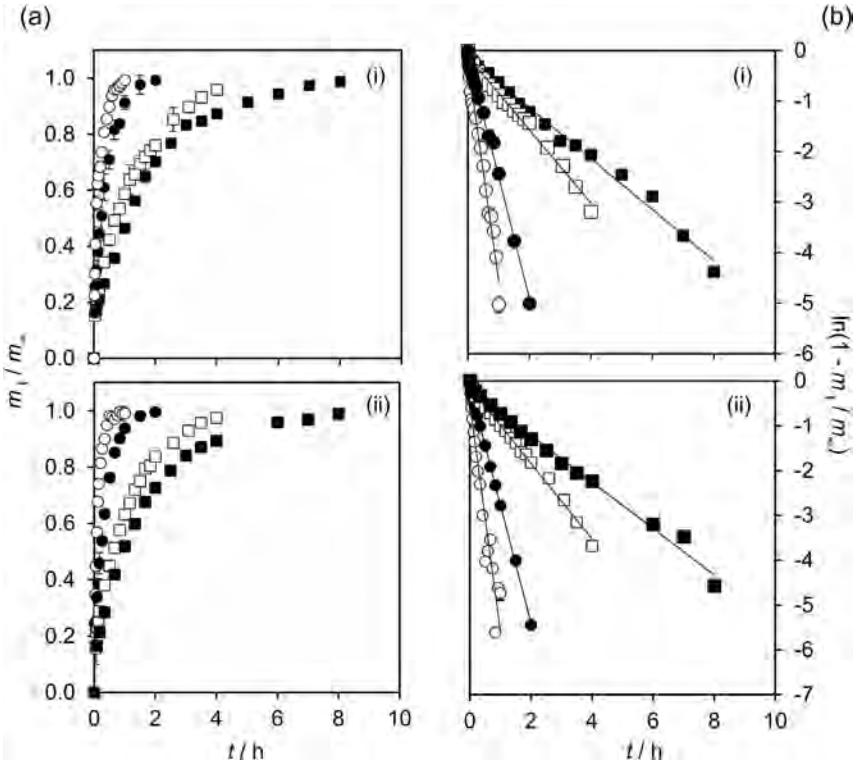

Fig. 1. Plots of: (a) the mass fraction m_t/m_∞ *versus* t, and (b) $\ln(1 - m_t/m_\infty)$ *versus* t for the release of thymol from: (i) PLA and (ii) PLA/kenaf films into 950 mL/L ethanol/water at: ■ 30 °C, □ 40 °C, ● 50 °C and ○ 60 °C where m_t is the mass of thymol released from the film at time t and m_∞ is the amount of thymol released from the film at equilibrium ($t = \infty$).

Table 1
Kinetic and the diffusion analyses for the release of thymol from PLA and PLA/kenaf composite films.

	Temperature/°C	First order kinetic analysis		SSE[a]	Diffusion analysis				Fickian diffusion	
					Short term	SSE	Long term	SSE		SSE
		$\nu_o \times 10^5$/g s^{-1}	$k_1 \times 10^5$/s^{-1}		$D \times 10^{12}$/m^2 s^{-1}		$k_2 \times 10^5$/s^{-1}		$D \times 10^{12}$/m^2 s^{-1}	
950 mL/L ethanol/water										
PLA	30	5.2	14.0	0.220	41	0.003	13.7	0.197	0.29	0.010
	40	7.3	20.1	0.111	93	0.002	20.0	0.057	0.60	0.060
	50	30.2	66.6	0.147	236	0.004	69.2	0.082	1.63	0.099
	60	49.9	119.6	0.569	1124	0.002	117.8	0.451	5.75	0.373
PLA/kenaf	30	5.4	14.4	0.254	90	0.001	13.8	0.194	0.56	0.001
	40	7.9	23.5	0.100	162	0.003	24.1	0.060	1.14	0.079
	50	38.8	73.2	0.036	443	0.002	73.2	0.009	3.10	0.065
	60	46.1	141.0	3.686	1340	0.000	124.4	2.720	10.05	0.498
150 mL/L ethanol/water										
PLA	60	0.8	4.8	0.086	28	0.001	5.2	0.053	0.17	0.006
	65	2.7	9.3	0.089	43	0.010	8.9	0.066	0.24	0.026
	75	12.3	46.0	0.325	132	0.003	45.9	0.248	0.66	0.072
	83	15.4	58.8	0.486	255	0.004	61.1	0.048	2.62	0.278
PLA/kenaf	60	1.8	7.0	0.395	75	0.002	7.2	0.327	0.40	0.010
	65	2.6	9.6	0.392	102	0.001	9.6	0.329	0.52	0.022
	75	10.0	36.7	0.460	195	0.001	35.9	0.373	0.90	0.098
	83	14.1	57.3	0.101	454	0.004	67.1	0.004	3.39	0.189

[a] SSE: Sum of squared errors.

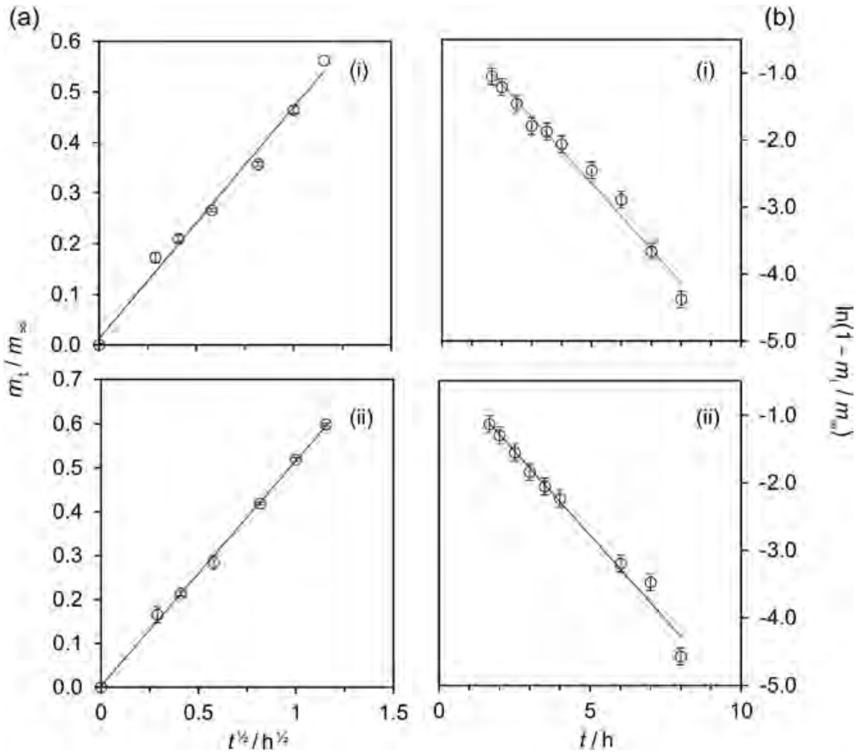

Fig. 2. Plots of: (a) mass fraction m_t/m_∞ *versus* $t^{1/2}$ and (b) $\ln(1 - m_t/m_\infty)$ *versus* t for the release of thymol from: (i) PLA and (ii) PLA/kenaf films into 950 mL/L ethanol/water at 30 °C where m_t is the mass of thymol released from the film at time t and m_∞ is the amount of thymol released from the film at equilibrium ($t = \infty$).

greater than 0.97.

The results in Table 1 confirm that the diffusion coefficients of thymol from the neat PLA and PLA/kenaf films into the 950 mL/L ethanol/water increase with increasing temperature. As expected, the diffusion rates of the composite films are higher than those obtained for the neat PLA films. This may be attributed in part to the presence of the kenaf fibre filler in the polymer matrix that creates voids thereby enabling the release of thymol from the film. These findings are consistent with the result obtained for the retention of thymol in a previous study whereby following processing the composite films were found to retain less thymol than neat PLA film (Tawakkal et al., 2015). It is important to note that the values of the *D* parameter found for the neat PLA and PLA/kenaf films containing thymol are one order of magnitude higher than those observed for active low-density polyethylene (LDPE), PBS and PP containing a similar AM agent and using 950 mL/L ethanol/water simulant (Cran et al., 2010; Ploypetchara et al., 2014; Ramos, Beltrán, Peltzer, Valente, et al., 2014a). Moreover, and as expected, active PLA films containing a non-volatile and thermally stable agent (i.e. resveratrol) prepared by Soto-Valdez et al. (2011) exhibit diffusion

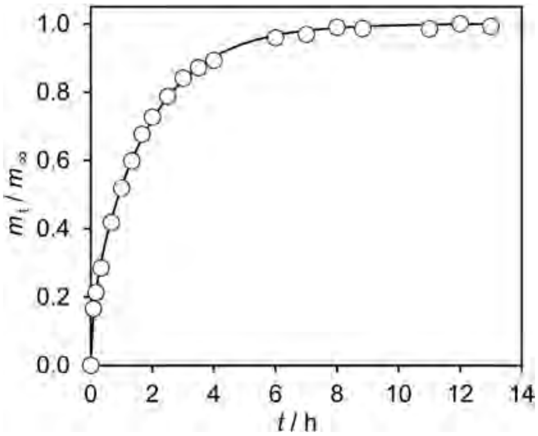

Fig. 3. Plot of the mass fraction m_t/m_∞ *versus* t for the release of thymol from the PLA film into 950 mL/L ethanol/water at 30 °C fitted using the Fick's law model where m_t is the mass of thymol released from the film at time t, and m_∞ is the amount of thymol released from the film at equilibrium ($t = \infty$).

coefficients that are one order of magnitude lower than those of PLA films containing a volatile AM agent such as thymol. Similar trends in the diffusion coefficients of thymol were observed for the neat PLA and PLA/kenaf films when immersed in the 150 mL/L ethanol/water simulant. However, the diffusion coefficients for the films immersed in the 150 mL/L ethanol/water simulant were consistently lower than those pertaining to the 950 mL/L ethanol/water simulant. The observed decrease in the diffusivity may be explained by the lack of affinity between thymol and water that leads to the limited solubility of thymol in water. Moreover, PLA is a hydrophobic polymer but ethanol is sufficiently non-polar to facilitate the swelling of the PLA matrix (Sato, Gondo, Wada, Kanehashi, & Nagai, 2013), dissolve the thymol and release it into the bulk of the simulant. According to Manzanarez-López, Soto-Valdez, Auras, and Peralta (2011), ethanol is also an aggressive solvent for PLA which can penetrate into PLA chains and release the active substance.

The lower concentration of ethanol in the 150 mL/L ethanol/water simulant may lead to a slight extent of swelling as well as hydrolysis of the PLA (Manzanarez-López et al., 2011). Interestingly this slight swelling of the PLA matrix appears to be more significant than the swelling effect of the kenaf fibres. The PLA/kenaf composites are semi-hydrophilic materials due to the presence of kenaf fibres that act as a hydrophilic filler. One may therefore expect the rate of diffusion of thymol from these composite systems (PLA/kenaf films) to increase as the water content of the simulant increases. Taib, Ramarad, Mohd Ishak, and Todo (2009) prepared PLA/kenaf composites containing polyethylene glycol (PEG) and reported that when the composite was immersed into the water, the fibres absorb water leading to expansion of the fibres. Such an effect may create internal stress in the adjacent matrix and resulting in the formation of microcracks. However, the rate of diffusion of thymol from the PLA/kenaf films was in fact found to be lower in the 150 mL/L ethanol/water simulant than in the 950 mL/L ethanol/water simulant. It can therefore be suggested that a complex and strong interaction exists between the kenaf, PLA and thymol in the composite systems. The PLA matrix may act as a coating agent to the kenaf fibre filler, preventing the water from swelling the kenaf fibres and releasing thymol that is associated with the fibres. Such an interaction between the PLA, kenaf fibre and thymol was reported previously where these composite systems were studied using Fourier transform infrared (FTIR) spectroscopic and thermogravimetric (TG) analyses (Tawakkal et al., 2015). In that study, the activation energy for the thermal release of thymol, using a 3D diffusion kinetic model, was found to be 46 kJ mol^{-1} in the case of neat PLA containing thymol and 65 kJ mol^{-1} for the PLA/kenaf composite system.

Fick's second law model was also used to calculate the diffusion coefficient by minimizing the sum of the squared errors (SSE) of the measured and calculated value (see Table 1). To determine the fit of the experimental data, Equation (5) was used and a non-linear regression function was applied to the data. Fig. 3 shows plots of m_t/m_∞ *versus* t for the diffusion of thymol from PLA/kenaf film into 950 mL/L ethanol/water at 30 °C where the data have been fitted using the Fick's second law model. The satisfactory fit of the experimental data suggests that the diffusion kinetics of thymol into 950 mL/L ethanol/water from the composite can also be adequately described by Fick's model. The SSE values for all the release models studied are also presented in Table 1. In general, the short-term diffusion model presents the best fit for the release kinetics with the lowest SSE values for all systems studied. This is followed by the Fickian diffusion model, with the first order model and long-term diffusion models showing some higher SSE values for some systems.

Using the diffusion coefficients obtained from the diffusion model, the effect of temperature on the diffusion coefficient for the release of thymol into 950 and 150 mL/L ethanol/water was determined and Arrhenius plots of the data are shown in Fig. 4. The activation energy for the diffusion process, E_a, was calculated from the slope of the ln(D) *versus* 1/T plot in each case (see Fig. 4) in accordance with the Arrhenius equation (see Equation (6)). The activation energies for the release of thymol from the neat PLA and PLA/kenaf films were found to be: 90.6 and 76.2 kJ mol^{-1} in 950 mL/L ethanol/water and 98.7 and 84.8 kJ mol^{-1} in 150 mL/L ethanol/water respectively. Significant differences were therefore found to exist amongst the E_a values for these active neat PLA and PLA/kenaf films immersed in the 950 and 150 mL/L ethanol/water simulants.

The E_a values for the neat PLA films are significantly higher than those found for PLA/kenaf films and this is attributed mainly to the strong interaction between the PLA and thymol that presumably is not as strong in the presence of the kenaf filler. Furthermore, the E_a values increase with an increase in water content of the simulant and this is consistent with the observations made in relation to the diffusion coefficients discussed above (see Table 1). The E_a values obtained in the present study are all considerably lower than those reported elsewhere for the diffusion of butylated hydroxytoluene (BHT) from PLA/BHT films into 950 mL/L ethanol/water which was 164.7 kJ mol^{-1} (Ortiz-Vazquez, Shin, Soto-Valdez, & Auras, 2011). This significant difference may be explained by the difference in molecular interaction and hydrogen bonding that exists between the polymeric matrix and the AM additive in these systems (Kuorwel et al., 2013). As expected, the trend in E_a values for the various systems when calculated using the Fick's law model are consistent with those calculated by the diffusion model given that the latter model has been derived from the former model. The activation energies using the Fick's law model for the release of thymol from the neat PLA and PLA/kenaf films were found to be: 83.3 and 80.9 kJ mol^{-1} in 950 mL/L ethanol/water and 118.5 and 96.3 kJ mol^{-1} in 150 mL/L ethanol/water respectively. These slight differences in the E_a values that were obtained using the different models might be due to the use of the diffusion coefficients derived from the short-term experimental data in the construction of the Arrhenius plot.

3.2. Film appearance

Fig. 5 shows the images of neat PLA and PLA/kenaf films after the release of thymol into 150 and 950 mL/L ethanol/water at the different temperatures studied. A considerable change in color for

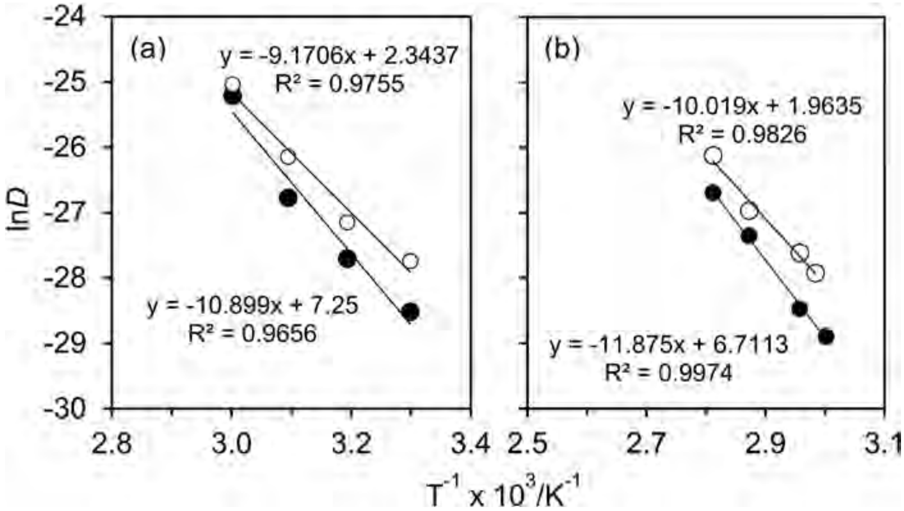

Fig. 4. Arrhenius plots of ln(*D*) *versus* 1/*T* for the release of thymol into: (a) 950 mL/L ethanol/water and (b) 150 mL/L ethanol/water from: ● PLA film and ○ PLA/kenaf film where *D* is the diffusion coefficient and *T* is the absolute temperature.

the neat PLA and PLA/kenaf films was observed after the release experiments. These color changes are due to the effects of temperature as well as the presence of water in the simulants. The color is observed to change from clear to opaque for the neat PLA immersed in 150 mL/L ethanol/water with an increase in opacity with increasing temperature. A similar observation was made in the case of the 950 mL/L ethanol/water simulant. The opacity of materials may be attributed to a polymer hydrolytic degradation process which can be related to crystallization of the PLA matrix as well as moisture absorption. The water molecules diffuse through the films, promoting hydrolysis leading to the formation of low molecular weight degradation by-products (Ramos, Beltrán, Peltzer, Dominici, et al., 2014b). In addition, color changes from brown to light brown are also observed for the composites immersed in 150 and 950 mL/L ethanol/water. For the PLA/kenaf composites immersed in 150 mL/L ethanol/water, the PLA

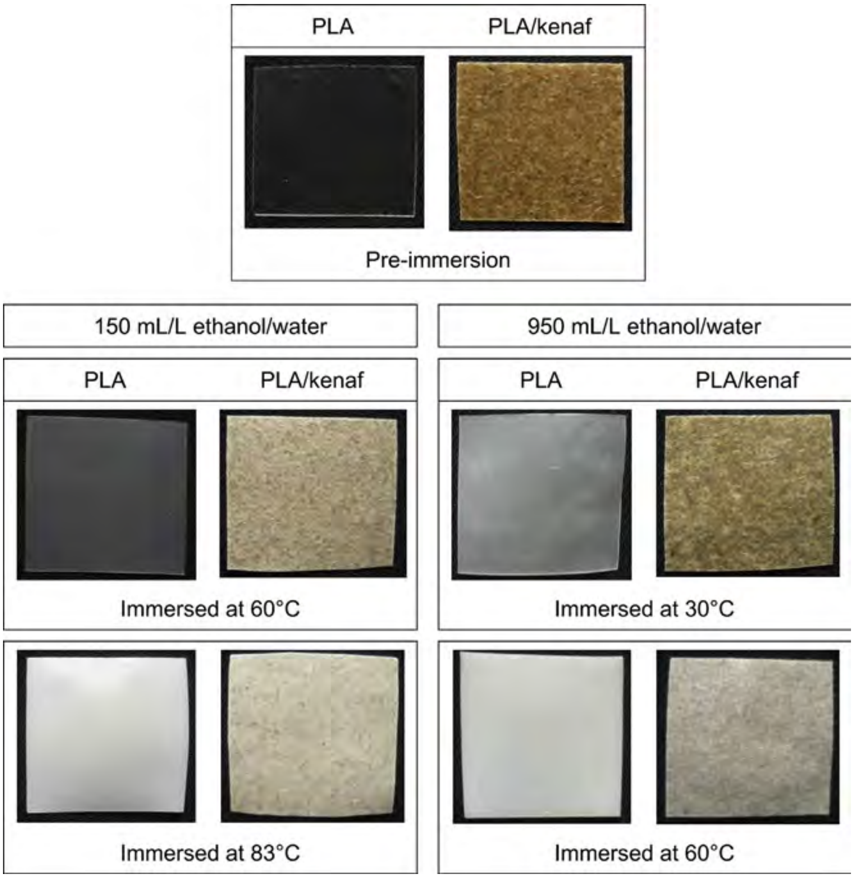

Fig. 5. Images of PLA and PLA/kenaf films after the release of thymol into 150 and 950 mL/L ethanol/water simulants at different temperatures.

surrounding the fibres dissolved or delaminated from the film surface revealing the kenaf fibers and this effect was less apparent in the 950 mL/L ethanol/water simulant.

4. Conclusions

The results of this study suggest that thymol is readily released from neat PLA and PLA/kenaf films into 150 and 950 mL/L ethanol/water simulants and the process can be described by an overall first-order kinetics model that can be used to determine the initial release rate. The short- and long-term diffusion models also adequately describe the release of thymol from these systems and the results are consistent with those obtained using a Fick's law analysis approach. The diffusion coefficient data suggest that the addition of the kenaf filler to the PLA matrix facilitates the release of thymol from the matrix and that the diffusion follows an Arrhenius relationship with temperature. Furthermore, an increase in polarity of the simulant that results from an increase in its water content decreases the propensity of thymol to be released from the composite system. Nonetheless, it is apparent that active PLA/kenaf composites containing natural AM agents such as thymol are potential candidates to be explored further for use as active packaging systems. Such systems have the advantage of being derived from natural sources, contain a naturally-derived AM agent and, as such, are expected to be more susceptible to biodegradation than their synthetically-based counterparts.

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Chapter 8 – Antimicrobial Activity and Storage Stability of Composite Films

Antimicrobial Activity and Storage Stability of Active PLA/Kenaf/Thymol Composites

Overview

The *in vitro* and *in vivo* analyses of AM activity of PLA and PLA/kenaf composite films against *E. coli* or inoculated on the surface of processed meat samples are discussed. The influence of thymol concentration and kenaf fibre loadings on the AM activity of the films are reported. Moreover, the retention of thymol during storage under different storage conditions is examined in this chapter. The capabilities of PLA and PLA/kenaf films containing thymol as an AM packaging systems for real foodstuff can be suggested from the results.

The manuscript entitled "Effect of Poly(Lactic Acid)/Kenaf Composites Incorporated with Thymol on the Antimicrobial Activity of Processed Meat." by Tawakkal I. S. M. A., Cran M. J. and Bigger S. W. has been accepted for publication in the Journal of Food Processing and Preservation.

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PART B:

DECLARATION OF CO-AUTHORSHIP AND CO-CONTRIBUTION: PAPERS INCORPORATED IN THESIS BY PUBLICATION

This declaration is to be completed for each conjointly authored publication and placed at the beginning of the thesis chapter in which the publication appears.

Declaration by [candidate name]: **Signature:** [redacted] **Date:** 07.12.2015

Intan S. M. A. Tawakkal

Paper Title:

Antimicrobial Activity and Storage Stability of Active PLA/Kenaf/Thymol Composites

In the case of the above publication, the following authors contributed to the work as follows:

Name	Contribution %	Nature of Contribution
Intan S. M. A. Tawakkal	70	Designed the experiment Performed the sample analyses Prepared major part of the manuscript
Marlene J. Cran	20	Contribution to writing of paper and journal submission
Stephen W. Bigger	10	Contribution to writing of paper

DECLARATION BY CO-AUTHORS

The undersigned certify that:

1. They meet criteria for authorship in that they have participated in the conception, execution or interpretation of at least that part of the publication in their field of expertise;
2. They take public responsibility for their part of the publication, except for the responsible author who accepts overall responsibility for the publication;
3. There are no other authors of the publication according to these criteria;
4. Potential conflicts of interest have been disclosed to **a)** granting bodies, **b)** the editor or publisher of journals or other publications, and **c)** the head of the responsible academic unit; and
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Signature 3	[redacted]	07.12.2015

Antimicrobial Activity and Storage Stability of Poly(Lactic Acid)/Kenaf/Thymol Composites

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8.1 Abstract

Bio-based composites comprised of poly(lactic acid) (PLA), kenaf fibres and thymol were developed and their antimicrobial (AM) properties and stability under different storage conditions investigated. The composite films containing 20-30% w/w thymol reduced *E. coli* in tryptone soy broth after two days at 37°C and imparted a significant zone of inhibition in contact with *E. coli* inoculated plates. The composite films also reduced *E. coli* inoculated on the surface of the processed sliced chicken samples after 30 days at 10°C both in direct contact and in the vapour phase. The thymol additive was retained in the PLA/kenaf films that were wrapped with aluminium foil after 3 months of storage at ambient temperatures, however unwrapped films lost some thymol to the atmosphere. The development of PLA/kenaf/thymol composite films shows a strong potential for the development of active packaging systems in order to extend the shelf life of some processed food products.

Keywords: antimicrobial activity, biopolymer, essential oil, natural fibres, food packaging

Practical Application: Packaging materials utilizing bio-based materials and a natural bio-active agent, thymol, to impart antimicrobial activity were developed. The presence of a natural bio-filler derived from kenaf fibres into a poly(lactic acid) matrix enhanced the release of thymol and consequently inhibited the growth of microorganisms. This can potentially extend the shelf life of some food products and can be further developed as a rigid packaging material and/or coating.

8.2 Introduction

Modern food packaging is designed to fulfil a range of functions in addition to providing a primary container for food products (Risch, 2009). One such function is to minimize the usage of preservatives added directly to food products in order to hinder foodborne illness outbreaks that may result from microbial spoilage (Han, 2005). Various forms of antimicrobial (AM) packaging have been implemented as alternatives to conventional preservatives to inhibit microbial growth in food, maintain the quality of the produce and, ultimately, to reduce food wastage (Qin *et al.*, 2015; Ramos *et al.*, 2013). There are several approaches used to integrate AM agents into polymeric materials including direct addition during processing, surface coating of formed articles, immobilizing AM agents by chemical grafting, and using polymers that possess intrinsic AM activity (Fernández-Pan *et al.*, 2015; Muriel-Galet *et al.*, 2013; Peretto *et al.*, 2014; Wang *et al.*, 2011). These techniques offer advantages over traditional preservatives, including the provision of continuous AM effect on foods for extended times and minimizing interactions and possible inactivation of AM agents with food components (Muriel-Galet *et al.*, 2012; Otoni *et al.*, 2014).

In addition to active food packaging, the utilisation of bio-based materials in food packaging applications such as edible films, flexible and rigid films and coatings has become increasingly popular in recent years (Otoni *et al.*, 2014; Peretto *et al.*, 2014). In this case, the perceived environmental benefits and the emerging economic pressures to reduce the dependence on fossil resources are the primary driving forces behind the growing interest in bio-materials (Kuorwel *et al.*, 2011; Peretto *et al.*, 2014; Petchwattana and Naknaen, 2015; Qin *et al.*, 2015). There is also an increasing consumer demand for natural, disposable, biodegradable and recyclable food packaging materials as well as demand for high-quality fresh food products (Rhim and Ng, 2007). Bio-based polymers such as poly(lactic acid) (PLA) have received much attention in the packaging research and development area over the past few decades. Most importantly, materials such as PLA can be synthesized from renewable and bio-derived monomers and are comparable to conventional petroleum based polymers in terms of their mechanical properties and processability (Jamshidian *et al*., 2010). Poly(lactic acid) is suitable for applications that involve delivery and release of additives and, under specific composting conditions, PLA is biodegradable (Auras *et al*., 2004). More recently, PLA has been widely used in advanced packaging applications such as antioxidant and AM films (Iniguez-Franco *et al*., 2012; Liu *et al*., 2009; Llana-Ruíz-Cabello *et al*., 2015; Qin *et al*., 2015; Wu *et al*., 2014b). With regard to the latter, the main focus has been directed towards the inhibition of targeted microorganisms by PLA-based materials containing a range of AM agents.

Although there are considerable benefits in using biopolymers for packaging applications, a major consideration is the current high cost of producing these materials (Jamshidian *et al*., 2010). The incorporation of natural fillers into biopolymers such as PLA is receiving attention, especially for the potential development of materials that have reduced environmental impacts with subsequently lower production costs. In particular, plant fibres such as wood, flax, jute, kenaf, hemp and sisal can be used as reinforcements and fillers in biopolymer materials. In addition to enhanced biodegradability, the resulting bio-composites can have improved mechanical strength and stiffness as well as reduced densities (Gurunathan *et al*., 2015; Liu *et al*., 2005; Tawakkal *et al*., 2012). Although there are many studies that suggest PLA is a suitable matrix for the incorporation of natural fibres in bio-composites (Awal *et al*., 2015; Rajesh *et al*., 2015; Tawakkal *et al*., 2012; Xia *et al*., 2015), there are limited reports on the combination of bio-based polymers with natural fibres and AM agents in packaging applications. In one example, the inhibition of *E. coli* in liquid media for PLA/triclosan film was enhanced when the film was incorporated with 10% w/w wood fibres (Prapruddivongs and Sombatsompop, 2012). Another study of a commercial lignin-based material Arbofill kokos®

containing short coconut fibres and 2% w/w thymol, a common AM agent, showed significant inhibition of Gram-positive *S. aureus* after 20 h incubation, although no AM activity against Gram-negative bacteria, *E. coli*, was observed (España *et al.*, 2012).

In a recent study of the interaction and quantification of thymol in PLA/kenaf films, it was suggested that the polymer may protect the AM agent during storage and the kenaf fibres may subsequently trigger the AM activity once exposed to a humid environment (Tawakkal *et al.*, 2016a). Clearly, the retention of volatile AM agents such as thymol as well as the activity of AM films can be influenced by different storage conditions and temperatures. Nevertheless, few studies have reported the loss of AM agents during film storage particularly for AM PLA-based materials containing natural fibres. This is an important consideration to ensure that adequate AM activity is maintained against target microorganisms following the production, transportation and storage of the packaging material. In this work, PLA biocomposites incorporated with kenaf fibres and the AM agent thymol were developed and the AM activities against *E. coli* were studied *in vitro* and on commercial deli chicken slice. The anti-fungal activity of the AM film on the surface of deli chicken slice and the retention of AM agent in the film during prolonged storage are also reported.

8.3 Materials and Methods

8.3.1 Materials

Polymer and kenaf fibre: Poly(lactic acid) (7001D Ingeo^{TM}; specific gravity 1.24; melting temperature 154°C) (Tawakkal *et al.*, 2014) was obtained from NatureWorks LLC, USA. Mechanically separated kenaf fibre (bast) was purchased from Ecofibre Industries, Australia. *Antimicrobial agent:* The thymol (T0501, purity of 99.5%) was a food-grade, Halal and Kosher certified product purchased from Sigma Aldrich Pty. Ltd., Australia. *Chemicals:* Sodium hydroxide and acetic acid were purchased from Merck Chemicals, Australia. *Media:* The media included tryptone soy broth (TSB) and nutrient agar purchased from Oxoid, USA, bacteriological broth obtained from Amyl, Australia, and 3M Petrifilm™ for *E. coli* enumeration purchased from 3M, USA. *Microorganism: Escherichia coli* (ATCC 8739) was obtained from the culture collection of Food Science Australia, Werribee, Victoria, Australia.

8.3.2 Production of PLA/Kenaf/Thymol Films

The composite films were prepared as outlined in detail in our previous work (Tawakkal *et al.*, 2014, 2016a). Briefly, the kenaf fibres were subjected to an alkali treatment, washed, neutralized and dried. The PLA polymer was also dried overnight prior to blending with thymol and kenaf fibers in an internal melt mixer. Following mixing, the composites were pressed into films for subsequent testing. The film formulations and the final retention of thymol in the films following thermal processing are shown in Table 8.1. The retention of thymol was determined using thermogravimetric analysis as reported in our previous work (Tawakkal *et al.*, 2016a).

Table 8.1 Percentage of thymol in PLA films after thermal processing as determined by thermogravimetric analysis.

Kenaf[#]/% w/w	Thymol[#]/% w/w	Thymol retained in films / % w/w
0	5	3.7 ± 0.2
30	5	3.1 ± 0.1
0	10	8.3 ± 0.2
10	10	7.6 ± 0.1
20	10	7.1 ±0.1
30	10	6.7 ± 0.1
40	10	6.8 ± 0.2
0	20	16.8 ± 0.3
20	20	13.8 ± 0.9
0	30	23.2 ± 1.5
20	30	19.7 ± 1.3

[#] Balance is PLA

8.3.3 Antimicrobial Activity of Films on Solid Media

The microorganism *E. coli* was selected to demonstrate AM activity *via* an agar disc diffusion assay (Kun and Marossy, 2013). The bacteria culture was stored in TSB broth containing 30% v/v glycerol at -80°C and was sub-cultured in broth twice before being used. Samples of 0.1 mL of *E. coli* suspensions containing approximately 10^6 - 10^7 CFU mL^{-1} were spread over a prepared agar surface in a Petri dish. Film discs of 6 mm diameter were placed in triplicate on the Petri dish containing the agar. Plates were incubated at 37°C for 24 h and the diameter of the resulting inhibition zone was measured directly after the incubation period using a digital Vernier calliper. The presence of a clear zone of inhibition around the test films was taken as indication of AM activity in the films and the test was performed in triplicate.

8.3.4 Antimicrobial Activity of Films in Liquid Media

The AM activity of films was tested in TSB liquid media. Cell cultures of *E. coli* in the stationary phase (optical density of *ca.* 1 at 600 nm) were diluted in fresh TSB to an optical density of 0.1 and grown for 1.5 h at 37°C until the growth reached the exponential phase (optical density of *ca.* 0.6 at 600 nm). The cells were then diluted into TSB (1:100) to obtain stock solution with a working concentration of *ca.* 10^5 CFU mL^{-1} (Shemesh *et al.*, 2015). At this stage, 0.1 mL of TSB containing the stock solution and 0.25 g of film were placed into separate sterile tubes with an additional 10 mL of TSB and were incubated at 37°C for 24 h. Serial dilutions were performed using 0.1% w/v peptone water and 1 mL of each final diluted mixture was placed on a 3M Petrifilm™ count plate. Colonies were counted after incubation at 37°C for 24 h and expressed in units of log CFU mL^{-1}. Controls comprising inoculated TSB in the absence of films were also tested and the bacteria enumeration was performed in triplicate.

8.3.5 Antimicrobial Activity of Films on Processed Food

Direct Food Contact Analysis

The AM activity of films on a real food product was tested. Processed sliced chicken with a thickness of *ca*. 0.2 cm was purchased from a local supermarket (Fettayleh Smallgoods). The samples were then placed on a sterile tray and were aseptically cut into 3 × 3 cm squares. The samples were inoculated on the upper surface with 0.1 mL of *E. coli* suspension prepared in accordance with Section 8.3.3. The inoculum was spread evenly over the surface of the chicken slice using sterile spreaders to obtain an initial coverage of *ca*. 5.0 log CFU cm^{-2}. After inoculation, the samples were placed inside a laminar flow cabinet for 30 min to facilitate bacterial attachment. Films of 5 × 5 cm were placed on the top of each inoculated sample which were then packed into sterile stomacher bags and sealed using a plastic bag sealer device. Inoculated samples in the absence of films were prepared as controls and all samples were stored at 10°C to simulate mild temperature abuse (Guo *et al*., 2015). On each sampling day, the samples were hand-massaged in 20 mL of 0.1% w/v peptone water for 1 min and placed in a laboratory paddle blender masticator for a further 1 min. Serial dilutions ranging from 10^1 to 10^7 were made in 0.1% w/v peptone water. Following this, 1 mL of each final diluted mixture was placed on 3M Petrifilm™ count plates and incubated for 24 h at 37ºC prior to enumeration. The final bacterial cell density was expressed in units of log CFU cm^{-2} (Guo *et al*., 2015).

Microbial Death Rate

The death rate of *E. coli* inoculated onto the deli chicken slice samples was measured in accordance with the procedure outlined by Bachrouri *et al*. (2002). In this procedure, equation (1) can be used to determine specific death rate, μ:

$$\ln(N) = \ln(N_0) - \mu t \tag{1}$$

where, N is the population surviving at any time t, and N_0 is the initial population. The specific death rate, μ, is obtained from the gradient of a plot of the equation (1) which was used to compare the AM activities of the films.

Data Analysis

The bacteria enumeration in CFU mL^{-1} and CFU cm^{-2} were transformed to $\log_{10}$ values and analysed using analysis of variance with SAS version 9.1 software (SAS Institute, NC, USA). A Student t-test analysis was used to determine significant differences between treatment means. A significant value was defined as one where $p < 0.05$.

Indirect Food Contact Analysis

Antimicrobial films with a constant diameter of *ca*. 25 mm were prepared in order to maintain the same equilibrium surface area of the films which were then attached to the lids of petri dishes containing untreated chicken slice to avoid direct contact with the samples and facilitate headspace release of the AM agent. The petri dish and film assembly were sealed with Parafilm™ and incubated at two temperatures, 10°C and 25°C, in duplicate. Samples without test films and samples that were in contact with films containing no thymol were also tested as controls. Images of the samples were taken every day for up to 18 days and were subjected to image analysis to determine the area of mould growth.

Image Analysis

The percentage of fungal coverage on the surface of the chicken samples was measured in order to investigate the antifungal activity of the AM films. Images of the samples in the form of black and white photographic JPEG files were analysed using original imaging algorithm software designed to systematically count the separate black and white pixel areas comprising the image and compile a normalized area count of the black pixels (i.e. fungal coverage). The

JPEG photographic standard stores images as a two-dimensional array of pixel information, each pixel having a red, blue and green (R, B, G) component along with other information associated with the pixel. The image processing algorithm firstly removes grey-scale shading from the image file by systematically examining the R, G, B information associated with each pixel and setting the pixel to either "white" ($R = 255$, $G = 255$, $B = 255$) or "black" ($R = 0$, $G = 0$, $B = 0$) in accordance with a threshold set by the user. The algorithm then counts the number of sequential black pixels in each line of the image array and compiles a count of these which can be expressed as a percentage of the total area of the image (i.e. the total number of pixels comprising the image).

8.3.6 Storage Stability of Thymol in AM Films

The release of thymol from the PLA and PLA/kenaf films was investigated under two different storage conditions: unwrapped and wrapped with aluminium foil. The unwrapped films were exposed to ambient conditions at *ca.* 24°C and 51% relative humidity (RH) whereas the wrapped films were stored under: (i) ambient conditions and (ii) refrigeration at 4°C and *ca.* 95% RH. The amount of thymol retained in the films was measured using a Perkin-Elmer Spectrum 100 Optica Fourier-transform infrared (FTIR) spectrophotometer (PerkinElmer, Boston, USA) equipped with a diamond crystal attenuated total reflectance (ATR) accessory. A total of 16 scans per run were conducted in the range of 4000-600 cm^{-1} at 4 cm^{-1} resolution. Samples were placed on the surface of the ATR crystal and clamped in place to maintain a consistent pressure and subsequent contact with the crystal. The peak area at wavenumber *ca.* 807 cm^{-1} which corresponds to the ring vibration of thymol was used to measure the amount of thymol retained in the films starting from day zero to day 90.

8.4 Results and Discussion

8.4.1 Antimicrobial Activity of Films *In-vitro*

Solid Media AM Activity

The PLA/thymol and PLA/kenaf/thymol films were tested *in vitro* in order to provide preliminary information about the potential AM activity against *E. coli*, which is a common microorganism found in perishable food products such as processed meats (Tao *et al*., 2014). Figure 8.1 shows a typical image of the AM effect of these films against *E. coli* after 24 h of incubation at 37°C. The results demonstrate that the PLA/thymol and PLA/kenaf/thymol films containing 30% w/w thymol imparted a strong AM effect against *E. coli* with a clear zone of inhibition observed directly below and around the test films. The clear zone indicates the inhibition of *E. coli* colonies caused by the release of thymol from the film to the agar medium. As expected, no inhibition of *E. coli* was evident for the control films containing no thymol.

The size of the inhibition zones was measured in order to investigate the effects of thymol concentration and kenaf fibre loadings of the AM films against *E. coli*. Figure 8.2 shows the average diameter of the inhibition zone following the incubation period for the PLA/thymol and PLA/kenaf/thymol film formulations. No zone of inhibition was observed for the films containing 5% w/w thymol, which is the lowest thymol concentration (data not shown). A similar investigation of the AM activity of solvent cast soy protein edible films enriched with oregano and thyme essential oils reported that the films containing 5% w/w thyme essential oil showed the more intense AM activity against *E. coli* compared with films containing oregano, with inhibition zone diameters of 49 and 45 mm respectively (Emiroglu *et al*., 2010). The solvent casted soy protein edible films containing thyme essential oil in this study were found to be effective in inhibiting the *E. coli* which is in contrast to the current studied films. The observed differences may be attributed due to the different type of polymeric materials and agents used, the method of film preparation as well as implementation of migratory and/or non-migratory system which influences the release and inhibition of the microorganisms. Generally, the thyme plant contains essential oils that are highly enriched with terpenoids, particularly monoterpenoid phenols such as thymol and carvacrol. Thymol (2-isopropyl-5-methylphenol), the agent used in the present study, is a naturally derived additive which is commercially available in a crystalline and colourless form.

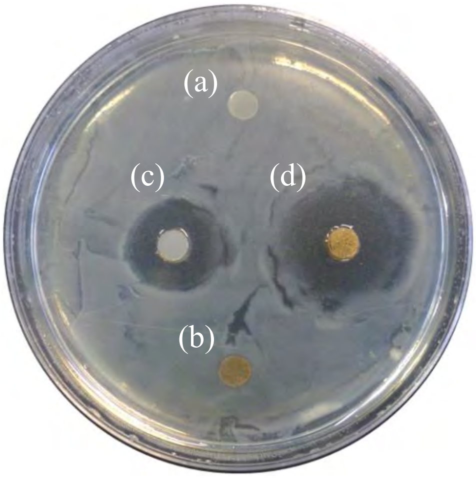

Figure 8.1 Antimicrobial activity of films against *E. coli* after 24 h of incubation at 37°C: (a) PLA control, (b) PLA with 20% w/w kenaf fibres, (c) PLA with 30% w/w thymol and (d) PLA with 20% w/w kenaf fibres and 30% w/w thymol.

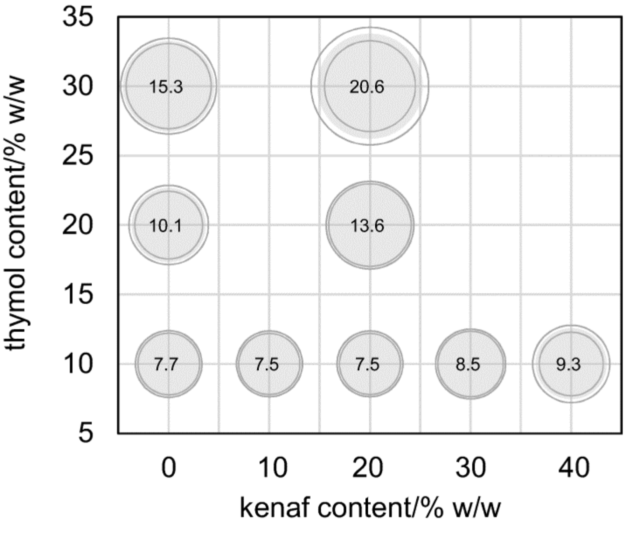

Figure 8.2 Influence of thymol and kenaf loadings on the zone of inhibition of PLA/thymol and PLA/kenaf/thymol films against *E. coli*. Solid lines represent error bars and the numbers represent the inhibition zone diameters in mm.

These current formulations may have a limited effect against *E. coli* at lower thymol content since the AM agent must diffuse from the polymer composite through to the agar medium (Suppakul *et al*., 2011). A similar MIC was reported for extruded poly(butylene succinate) films containing 10% w/w thymol tested *via* the agar diffusion method (Petchwattana and Naknaen, 2015). In another study, the zone of inhibition in extruded polypropylene films containing 8% w/w thymol showed negligible zone of inhibition against *E. coli* although no growth was observed directly under the film (Ramos *et al*., 2012). This finding is in accordance with the MIC of 10% w/w thymol suggested in the present study.

As shown in Figure 8.2, it is clear that increasing the concentration of thymol from 10 to 30% w/w increased the area of the inhibition zone and this effect is further enhanced with the addition of more kenaf fibers. For the films containing 10% w/w thymol, a small increase in the inhibition zone was observed with increasing kenaf loading. The PLA/kenaf/thymol film containing the highest kenaf fibre content (40% w/w) exhibiting a *ca*. 10% increase in the size of the zone of inhibition compared with the film containing the next highest kenaf loading (30% w/w). The higher loading of kenaf fibres in the film may have increased the hydrophilicity of the film resulting in water molecules being absorbed at the surface of the film which may contribute to a potential burst release of the AM agent which may also have an effect over the long term release profile. It is also clear from the results shown in Figure 8.2 that, for a given kenaf loading, increasing the thymol loading has a marked effect on the AM activity of the film. For example, at a constant kenaf loading of 20% w/w, increasing the concentration of thymol from 10 to 30% w/w produces a concomitant *ca*. three-fold increase in the diameter of the zone of inhibition.

Liquid Media AM Activity

The AM activity of PLA/thymol and PLA/kenaf/thymol films containing 10% w/w thymol and 30% w/w kenaf fibers against *E. coli* in a liquid media is represented in Figure 8.3(a). The results show that the concentration of *E. coli* in the presence of PLA films containing 10% w/w thymol was similar to those of the control (i.e. inoculated TSB that was not in contact with a test film) after 24 h, indicating that the PLA/thymol film showed little inhibitory effect on the growth of *E. coli*. After 48 h of incubation, the PLA/thymol film slightly reduced the cell count compared with the control sample, suggesting that thymol could diffuse through the polymer matrix of the film after an extended period of time (Nostro *et al*., 2007). After 24 h of incubation the composite film containing 30% w/w kenaf fibres and 10% w/w thymol showed a reduction in the *E. coli* population of *ca.* 1 log CFU mL^{-1} compared to both the control and PLA/thymol film. In addition, no recovery of injured bacteria cells was observed after 48 h incubation for both films containing thymol. It is clear that the PLA/kenaf/thymol film imparted a significant inhibitory effect compared to the PLA/thymol film, which may be attributed to the higher release rate of thymol from the composite film in the liquid media.

The presence of kenaf fibres in the polymer matrix may create voids thereby leading to the release of thymol (Tawakkal *et al*., 2016b). This finding is consistent with the observation made in relation to previous scanning electron microscope images where visible voids in the composite materials are shown as well as the diffusion rate of thymol from the PLA/kenaf films into fatty and aqueous food stimulants (Tawakkal *et al*., 2014; Tawakkal *et al*., 2016b). Similar results have also been observed in other systems whereby it was suggested that the presence of larger micro-voids in solvent-cast films of PLA/poly(ε-caprolactone) (PCL) containing thymol facilitates the release of the AM agent to the surface of the film and improves its AM activity against *E. coli* after 24 h in liquid media (Wu *et al*., 2014a). Moreover, hydrophilic fibres can swell when immersed in liquid media resulting in internal stresses generated in the adjacent polymer matrix that may produce microcracks, further facilitating the release of thymol (Tawakkal *et al*., 2016b).

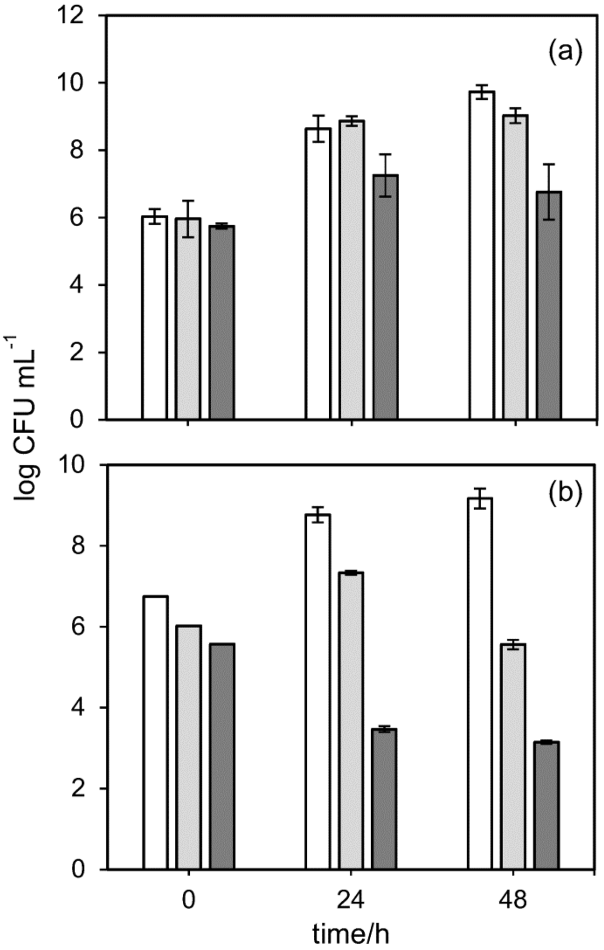

Figure 8.3 Liquid media antimicrobial activity of PLA formulations (a) control; PLA containing 10% w/w thymol; PLA containing 10% w/w thymol and 30% w/w kenaf fibres; (b) control; PLA containing 30% w/w thymol; PLA containing 20% w/w kenaf fibres and 30% w/w thymol: (□) PLA control, (░) PLA/thymol and (▓) PLA/kenaf/thymol films.

The results in Figure 8.3(b) show the inhibitory activity of films at higher thymol concentration (30% w/w) against *E. coli* in liquid media where the kenaf loading was 20% w/w in the PLA/kenaf composites. In this case, the higher concentration of AM agent in the films reduces the population of *E. coli* in the liquid media almost immediately upon contact which may be due to the thymol that is present on the surface of the film. At this level of thymol, only a moderate extent of AM activity was observed in the active PLA/thymol film up to 48 h incubation period suggesting that a significant interaction persists between the PLA matrix and thymol, thus preventing the liquid media from effectively swelling the film and releasing the thymol (Qin *et al.*, 2015). This may be attributed to the selective adsorption as well as possible encapsulation of thymol in the polymer matrix. Figure 8.3(b) further suggests that when kenaf fibres are introduced, the composite film exhibits significant AM activity by suppressing the growth of *E. coli* at 24 h. This film reduces *E. coli* by *ca*. 2 log CFU mL^{-1} after 24 h with no recovery of injured bacteria cells at 48 h. This finding is consistent with the results of the agar disc diffusion tests (see Figure 8.1). In a similar study, it was found that a higher loading of wood flour particles resulted in the release of more triclosan onto the composite surface and a subsequent inhibition of the growth of *E. coli* in a liquid medium (Prapruddivongs and Sombatsompop, 2012). Wu *et al.* (2014a) found that the AM activity of PLA/PCL films containing up to 12% w/w thymol were higher against Gram-positive bacteria (*L. monocytogenes*) than Gram-negative bacteria (*E. coli*) due to differences in the structures of the cell walls (Wu *et al.*, 2014a). Moreover, Del Nobile *et al.* (2008) prepared zein films incorporated with thymol from 10 to 35% w/w loading and found that all the films effectively inhibited the growth of *Pseudomonas* spp. (Del Nobile *et al.*, 2008).

8.4.2 Antimicrobial Activity on Real Foods

Direct Contact AM Activity

To further evaluate the efficacy of the active PLA composites, samples were tested in contact with a real food sample, namely deli chicken slice. Table 8.2 shows the population counts of *E. coli* in log CFU cm^{-2} on the surface of the inoculated chicken samples as a function of storage time at 10°C for each of the AM films. The results in Table 8.2 confirm that the population of

E. coli decreased upon increasing the thymol concentration from 10 to 30% w/w. No significant differences were observed between the bacteria counts for PLA films containing 10% w/w thymol and the control (i.e. inoculated chicken sample that was not in contact with a test film) in the first 13 days. As expected, the AM activities of the PLA/kenaf/thymol films are higher than those obtained for the PLA/thymol films, with an inhibitory activity against *E. coli* that is sustained for *ca*. 4 weeks. These findings are consistent with the results obtained in the previous *in vitro* studies (see Figure 8.2 and Figure 8.3), with the AM agent in the PLA/kenaf/thymol films found to have been activated or released almost instantaneously once the film is in direct contact with the substrate. Guo *et al*. (2015) prepared edible films based on chitosan loaded with different AM agents and found that the films reduced *Listeria* in a deli chicken slice to 3.7 log CFU cm^{-2} after 5 weeks at 10°C, with control samples reaching over 6.6 log CFU cm^{-2}. Emiroglu *et al*. (2010) found that isolated soy protein films containing thyme essential oil reduced coliform counts to 5 log CFU g^{-1} in ground beef patties at 4°C during 12 days of storage.

Table 8.2 Antimicrobial activity of PLA and PLA/kenaf films against *E. coli* on chicken slice stored at 10°C for 30 days.

		E. coli counts/ log CFU cm^{-2}				
Kenaf$^{\#}$/% w/w	Thymol$^{\#}$/ % w/w	Day 2	Day 6	Day 13	Day 19	Day 30
†0	0	4.42b^{c}	4.30^{b}	4.15^{b}	4.13^{a}	3.64^{a}
0	10	4.49^{bc}	4.75^{a}	4.32^{a}	3.87^{b}	3.55^{ab}
0	20	4.55^{ab}	3.83^{c}	3.59^{d}	3.33^{c}	3.09^{b}
0	30	4.44^{bc}	3.73^{c}	3.10^{f}	2.64^{d}	2.09^{c}
30	10	4.72^{a}	4.24^{a}	3.80^{c}	3.74^{b}	3.10^{b}
20	20	4.27^{c}	3.77^{c}	3.32^{e}	2.71^{d}	2.39^{c}
20	30	3.19^{d}	2.45^{d}	1.22^{g}	ND	ND

$^{\#}$ Balance is PLA; ^{abc} values are expressed as mean ± standard deviation, different letters in the same column indicate significant difference ($p < 0.05$); ND = not detected; †control sample without films

The death rates of *E. coli* in the presence of the PLA/thymol and PLA/kenaf/thymol formulations were determined for all systems and are plotted in Figure 8.4 as a function of the concentration of thymol in the formulation. The death rates were determined from the gradients of plots of the logarithm of the surviving population *versus* time (see equation (1)), the data having been derived from Table 8.2. Figure 8.4 shows that the death rate of *E. coli* in the PLA/thymol and PLA/kenaf/thymol films increased with increasing thymol concentration. The latter films showed significantly higher death rates than the PLA/thymol films, consistent with the previous observations, with the PLA/kenaf/thymol film containing 30% w/w thymol demonstrating the strongest inhibitory effect on the growth of *E. coli* on the chicken samples with a death rate of *ca*. 0.19 day^{-1}. The results also suggest that the incorporation of thymol into PLA and PLA/kenaf films prevents the recovery of injured bacterial cells for up to 30 days and this may be attributed to the strong AM properties of thymol.

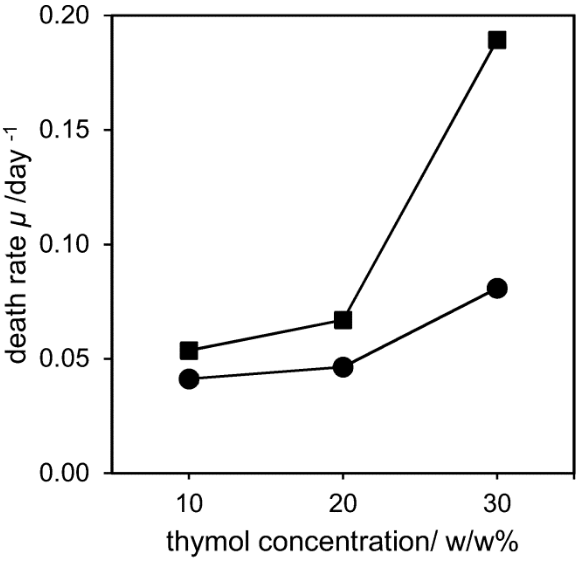

Figure 8.4 Death rate of *E. coli* inoculated on deli chicken slice and stored at 10°C in contact with: (●) PLA/thymol and (■) PLA/kenaf/thymol composite films.

Indirect Contact AM Activity

The coverage of fungi on the surface of chicken samples was measured *via* visual observation together with semi-quantitative image analysis and the antifungal activity of all films was tested using a vapour phase method. Figure 8.5 shows the appearance of chicken samples placed in petri dishes containing AM PLA and PLA/kenaf films at 25°C for up to 6 days. The images show that fungal growth commences with the appearance of white cottony mycelium at day 4 which becomes more apparent on day 6, with green sporulating mould clearly observed. Fungi such as moulds that are commonly found on meat and poultry include *Aspergillus, Penicillium, Rhizopus, Mucor, Cladosporium, Geotrichum, Thamnidium* among others (Pitt and Hocking, 2009). Moulds also can grow on refrigerated meat, where it can be detected by rot spots of various sizes and colours, unsightly scabs, slime, white cottony mycelium or highly coloured sporulating mould (Pitt and Hocking, 2009).

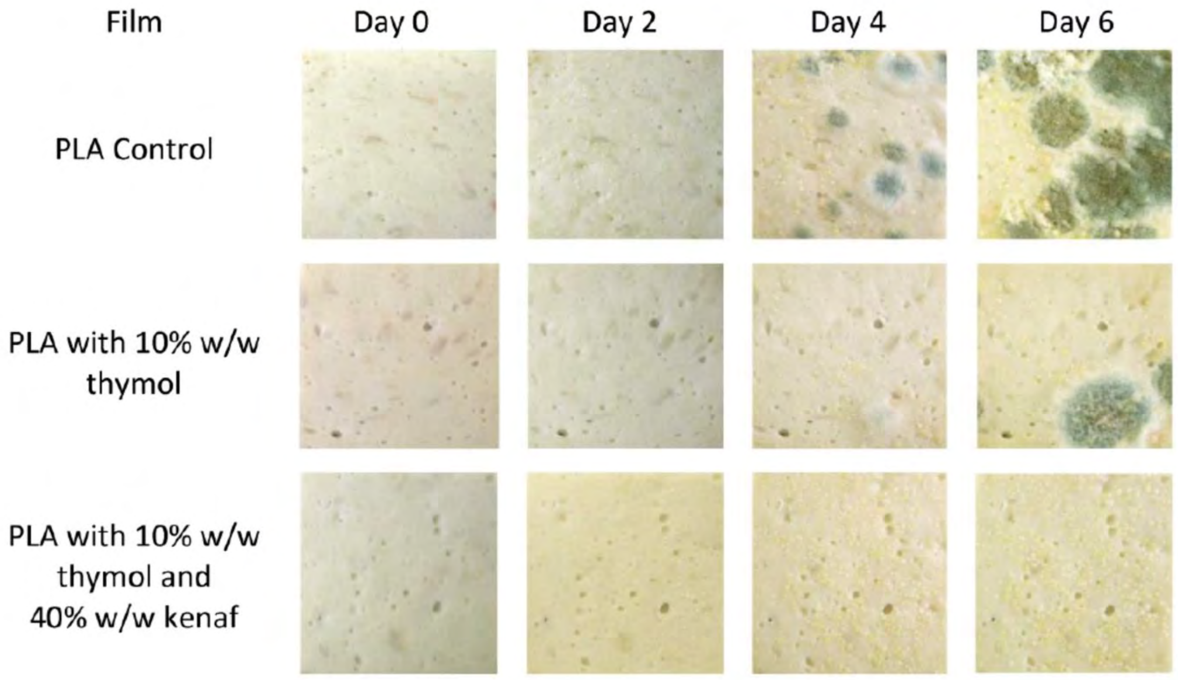

Figure 8.5 Fungal coverage on the surface of deli chicken slice samples stored at 25°C in the presence of selected AM film formulations.

Figure 8.6(a) and (b), show that the percentage of fungal coverage per unit area (f) gradually increased during the storage period at storage temperatures of 10 and 25°C. In the case of the control sample and samples that were in contact with films without thymol, a rapid growth of fungus was observed starting at day 4 whereas for samples in contact with the active films, no fungal growth was observed until day 6 at 25°C. The presence of fungus was first observed on the chicken samples stored at 10°C after 12 days of storage. Under both storage conditions, it was observed that PLA/thymol and PLA/kenaf/thymol films containing high thymol concentrations of 20 and 30% w/w completely inhibited fungal growth during the storage period (results not shown). Although the headspace was not measured in the current study, this result may be attributed to the release of a large amount of thymol from the active films into the headspace (Del Nobile *et al*., 2008).

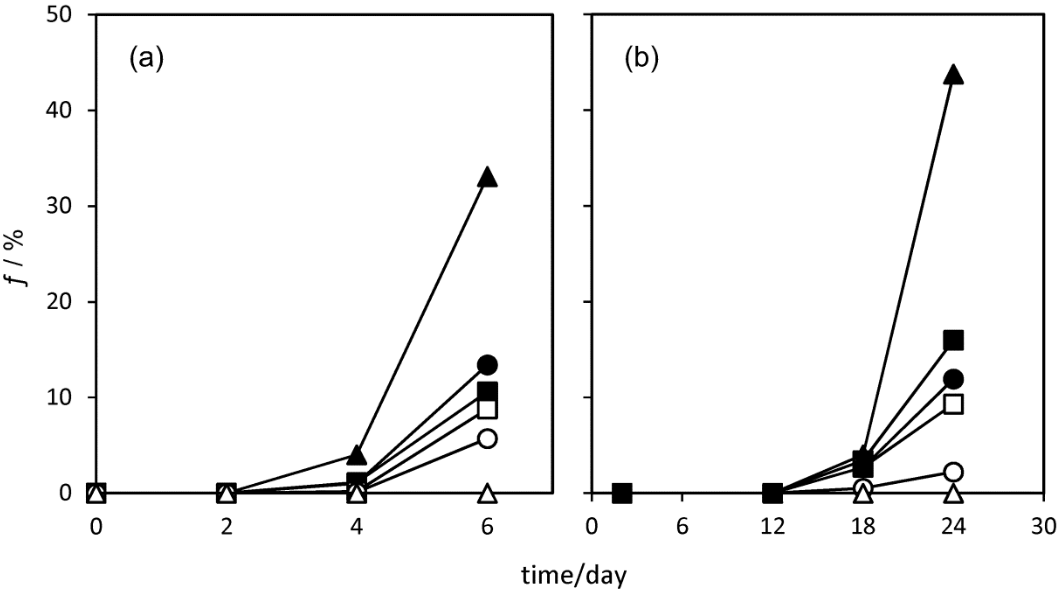

Figure 8.6 Percentage of fungal coverage per unit area (*f*) of deli chicken slice samples stored at: (a) 25°C and (b) 10°C. Key: ▲ control, ■ neat PLA film, ● PLA/kenaf film containing 30% w/w kenaf fibres, □ PLA/thymol film containing 10% w/w thymol, ○ PLA/kenaf/thymol film containing 10% w/w kenaf fibres and 10% w/w thymol and ∆ PLA/kenaf/thymol film containing 40% w/w kenaf fibres and 10% w/w thymol.

In general, the results show that the PLA/thymol and PLA/kenaf/thymol films containing 10% w/w thymol and lower kenaf fibres loading (10% w/w) are slightly better at retarding fungal growth than the control samples (see Figure 8.6(a) and (b)). However, active PLA films filled with a high loading of kenaf fibres (such as 40% w/w) and containing 10% w/w thymol demonstrated significant antifungal activity at both storage temperatures. The latter finding is consistent with the observed AM activity of PLA/kenaf/thymol films in contact with the solid agar media (see Figure 8.2). Muriel-Galet *et al.* (2015) tested the AM activity of ethylene vinyl alcohol (EVOH) films containing oregano essential oil and green tea extract on the fungal species *Penicillium expansum via* the agar diffusion method in the vapour phase. It was revealed that the EVOH films presented a strong effect against fungal growth after 12 days of storage at 30°C. The results obtained from the current study also provide evidence of the effectiveness of volatile AM agents when incorporated in PLA containing natural fibres at improving the shelf-life of perishable food. Further evaluation of the effectiveness of the active films against other microorganisms, on various real-food products, and at different storage temperatures could also be considered for future studies.

8.4.3 Effect of Different Storage Conditions on Thymol Retention

Although many studies have focused on the release of thymol from the film into food simulants, few have investigated the release of thymol into the atmosphere or surroundings as a function of storage time and temperature. In one such example, Suppakul *et al.* (2011) reported that there was no difference in AM activity of the AM LLDPE and films against *E. coli* at the time when the film was originally produced and after 1 year of storage, although a reduction in additive concentration was detected. In the present study, the release of thymol from the films was determined by measuring the decrease of the FTIR band at 807 cm^{-1} over time (Sanchez-Garcia *et al.*, 2008). According to Tawakkal *et al.* (2016a), the most intense peaks at 738 and 807 cm^{-1} are assigned to ring vibrations of thymol. Figure 8.7 shows the normalized peak area of thymol in the PLA/thymol and PLA/kenaf/thymol films containing 10% w/w thymol under different storage conditions for up to 3 months. The peak area of thymol at *ca.* 807 cm^{-1} on day one to day 90 was referenced to the peak area of thymol on day zero in order to normalize the peak area.

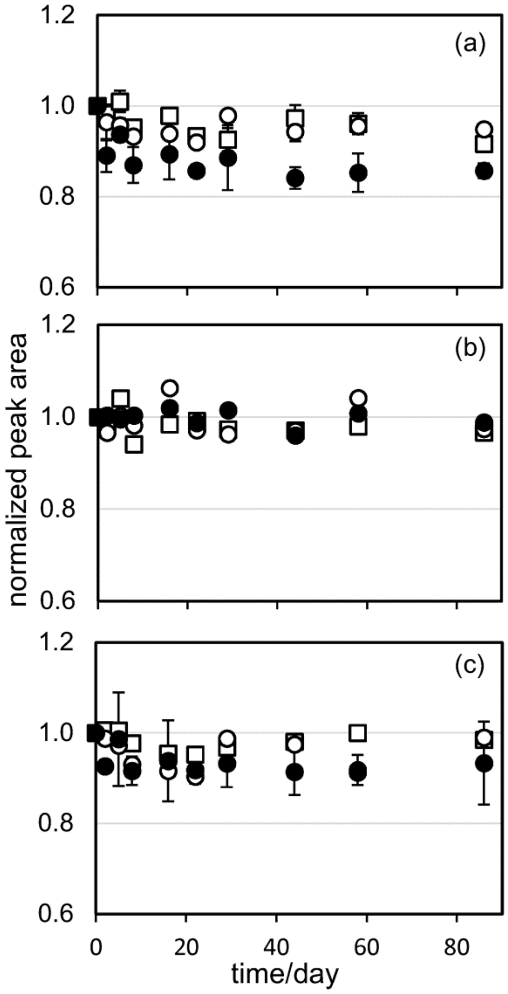

Figure 8.7 Normalized thymol peak area in films up to 3 months of storage under different storage conditions: (a) films unwrapped in aluminium foil and stored at ambient temperature, (b) films wrapped in aluminium foil and stored at ambient temperature and (c) films wrapped with aluminium foil and stored at 4°C. Key: □ PLA/thymol film containing 10% w/w thymol; ○ PLA/kenaf/thymol film containing 10% w/w kenaf and 10% w/w thymol and ● PLA/kenaf/thymol film containing 30% w/w kenaf and 10% w/w thymol.

As shown in Figure 8.7(a), the unwrapped PLA/kenaf films containing 30% w/w kenaf loading showed a higher loss of thymol when stored under ambient conditions compared with those formulations with a lower kenaf loading. The results also suggest that most of the loss of thymol from the PLA/kenaf films containing the higher kenaf loading occurs during the first 8 days of storage and the films achieved an equilibrium thymol concentration after *ca.* 16 days of storage. In a few cases, the concentration of thymol was detected to be more than the normalized value and this may be attributed to the distribution/dispersion of thymol in the surface of pressed films and subsequent migration of thymol to the surface. It is important to note that the measurement of thymol concentration by using FTIR detects thymol in relation to the surface of the pressed films with the infrared beam penetrating only a few microns below the surface. Moreover, the release of thymol from the PLA/kenaf films exposed to the atmosphere may be attributed to its high volatility as well as the presence of voids that facilitate the release of the agent from the films. This is consistent with the micrograph images presented in a previous study where voids and loose fibers are observed on the fracture surface of PLA/kenaf composites containing 30% w/w kenaf loading (Tawakkal *et al*., 2014).

For the foil wrapped samples stored at ambient and refrigeration temperatures (see Figure 8.7(b) and (c) respectively), the thymol concentration was found to be more stable in the PLA and PLA containing 10% w/w kenaf loading films, with *ca.* 85% thymol retained in these films compared with the films stored in the open environment. As expected, the PLA and PLA/kenaf films wrapped in the aluminium foil retained the thymol and a similar observation was found by Kuorwel (2011) who prepared starch films incorporated with thymol. Interestingly, for the PLA/kenaf/thymol films containing the higher fibre loadings, a slight loss of thymol was observed for these films when wrapped in the aluminium foil and stored at 4°C. This may be attributed to the presence of surface moisture on the film created by condensation that may remove some of the AM agent on the surface when the film sample is prepared prior to the FTIR measurement. In addition, the hydrophilicity of the kenaf fibres may enable the condensate to swell the film and facilitate the thymol release to the environment. This observation suggests that in any commercial application of these formulations, a protective barrier layer and/or hermetic seal of the films should be implemented to avoid condensation and subsequent loss of AM agent to the atmosphere during storage in order to preserve the initial AM activity at the time of manufacture.

8.5 Conclusion

The AM efficacy of PLA films incorporated with kenaf fibers and thymol were tested against *E. coli* and naturally occurring fungi. The PLA/thymol and PLA/kenaf/thymol films containing high thymol concentrations showed a strong AM activity against *E. coli* with a significant inhibition of the growth in both solid and liquid media. The composite films also significantly reduced the population of *E. coli* on inoculated chicken slice samples when placed in direct contact with the food. It is suggested the films retarded natural fungal growth in the chicken slice samples *via* the release of thymol into the headspace surrounding the samples. The PLA/thymol and PLA/kenaf/thymol films containing higher thymol content as well as higher kenaf loading were more effective in controlling or limiting the fungal growth than those containing lower loadings of these additives. After storage for 3 months at ambient temperatures, a slight decrease in the retention of the AM additive was observed in PLA/kenaf/thymol films containing 30% w/w kenaf and 10% w/w thymol that were not wrapped with aluminium foil.

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Chapter 9 – Conclusions

9.1 General Conclusions

Thymol can be successfully incorporated into the commercially available biopolymer PLA as well as PLA composites containing kenaf fibres to produce active packaging materials that exhibit activity against *E. coli* both *in vitro* and on the surface of a real food product. The mechanical, thermal, morphological, and biodegradation properties of PLA and PLA/kenaf composites containing thymol were successfully characterized. In addition, the optimization of material formulations and properties such as the effects of different kenaf fibre loadings and thymol concentrations was achieved. In this chapter, conclusions are drawn based on the discussion of the results presented in Chapters 3 to 8.

9.1.1 Effect of Kenaf Fibre Surface Treatment

In general, the presence of thymol in PLA and PLA/kenaf composites containing 30% w/w UK and TK respectively imparted a plasticizing and/or lubricating effect. A relatively weak adhesion between the PLA matrix and the fibres was confirmed microscopically in composites containing thymol. The tensile strength of PLA composites containing TK fibres was slightly higher than that of composites containing UK fibres suggesting the alkaline treatment imparts a reinforcing effect within the polymer matrix. The incorporation of 10% w/w thymol into the composites decreased the tensile strength and stiffness irrespective of the fibre pre-treatment with no significant changes to the elasticity. Thermal analysis by DSC showed a general decrease in T_g, T_{cc}, and T_m in PLA and PLA composites containing 10% w/w thymol compared with the formulations without thymol, suggesting changes in the phase structure of the polymer. The TG decomposition temperature of the PLA composite containing TK fibres and 10% w/w thymol was slightly increased indicating an increase in the thermal stability compared with the PLA composite containing the UK fibres. Under controlled composting conditions, the degradation of the PLA and PLA containing TK fibres progressed rapidly resulting in a complete loss within 48 days.

9.1.2 Effect of Kenaf Loadings and Thymol Concentrations

Further characterisation of PLA and PLA/kenaf composites containing TK was systematically performed in order to investigate the effects of the natural filler kenaf as well as the AM additive thymol on the stability and performance of the material during processing. The incorporation of thymol into PLA/kenaf composites decreased the tensile strength with no significant further changes observed upon increasing the kenaf fibre loading. The tensile strength and stiffness of composites however increased with higher fibre loadings, thereby imparting a reinforcement effect within the PLA composite. It was noted that an increased loading of kenaf fibres in the PLA resulted in a decrease in the TGA decomposition temperature indicating a reduced thermal stability of the PLA whereby some portion of the polymer is replaced with less thermally stable fibres in the composite materials. These results are in agreement with the results of kinetics analysis using two algorithms described in Chapter 5 where the PLA and PLA composite each decomposes in accordance with the contracting volume (R3) model and where the addition of the kenaf filler to the PLA destabilizes the polymer and lowers the apparent activation energy from 190 kJ mol^{-1} to *ca.* 150 kJ mol^{-1}. The model identification algorithm exhibits significant promise as a method by which applicable kinetic models can be identified. Moreover, the developed kinetic approach was used as a tool to investigate the interactions between the thymol, kenaf fibres and PLA of the composite systems.

9.1.3 Interaction and Retention of Thymol

The FTIR analysis of the PLA and PLA/kenaf composites containing thymol showed that the thymol interacts with PLA and kenaf as revealed by significant shifts in the various FTIR absorption bands. Active PLA/kenaf composites retain less thymol upon processing than PLA alone and the composites containing the highest fibre loadings demonstrated the lowest retained thymol content. Nevertheless, the apparent activation energy calculated from the kinetic model for thymol release from the PLA/TK composites was found to be greater than that for the release of thymol from PLA alone, which is consistent with the intermolecular attractions that occur as a result of hydrogen bonding between the components in the composite. It would therefore appear that the disruption to the crystalline regions caused by the addition of kenaf, along with the concomitant creation of voids and the resulting decrease in tortuosity, facilitate the release of thymol from the composite. In addition, the increased hydrophilicity due to water absorption into the created voids may increase the migration of water into the polymer matrix and potentially increase the creation of further voids due to an internal autocatalytic effect.

9.1.4 Migration of Thymol

It is suggested that thymol is readily released from PLA and PLA/kenaf films into 15% and 95% v/v ethanol/water simulants and the process can be described by an overall first-order kinetics model that can be used to determine the initial release rate. The short- and long-term diffusion models also adequately describe the release of thymol from these systems and the results are consistent with those obtained using a Fick's law analysis approach. The diffusion coefficient data suggest that the addition of the kenaf filler to the PLA matrix facilitates the release of thymol from the composite and that the temperature dependency of diffusion follows an Arrhenius relationship. As expected, the release rate of thymol from the PLA film formulations into fatty and aqueous food simulants was found to be temperature-dependent. Furthermore, an increase in polarity of the simulant that results from an increase in its water content decreases the propensity of thymol to be released from the composite system.

9.1.5 Antimicrobial Activity and Storage Stability

The PLA and PLA/kenaf films containing high thymol concentrations showed a strong AM activity against *E. coli* with a significant inhibition of the growth in both solid and liquid media. The PLA/kenaf/thymol composite films significantly reduced the population of *E. coli* on inoculated chicken slice samples when placed in direct contact with the food compared with the PLA/ thymol films. It is suggested that the films containing higher thymol content as well as higher kenaf loading were more effective in controlling or limiting the fungal growth in the chicken slice samples *via* vapour phase transmission of the AM agent than those containing lower loadings of these additives. Moreover, compared to samples that were wrapped in aluminium foil prior to storage, a slight decrease in the retention of the AM additive was observed in PLA/kenaf/thymol films containing 30% w/w kenaf and 10% w/w thymol that were not wrapped in the foil after storage for 3 months at ambient temperatures.

9.2 Significance of the Findings

Nowadays, there is increasing consumer demands for fresh, high-quality and natural foods with minimum amounts of preservatives that are packaged in environmentally friendly materials that prolong the shelf life of the foodstuff. The development of bio-based materials that are renewable and biodegradable is of increasing priority. The use of bio-fillers incorporated into PLA is receiving attention mainly for the potential these to create materials that are more environmentally sustainable than currently used materials. The results of the current study suggest the use of kenaf fibres as a natural filler or reinforcement in PLA composites will enable the production of a low-cost AM packaging material that is environmentally sustainable due to the renewability and abundance of the associated raw materials. The incorporation of natural fibres into bio-based polymers together with the addition of naturally-derived AM agents that have minimal impact on the environment is likely to lead to the next generation of packaging materials.

The present study has demonstrated that the active PLA composites containing natural filler have acceptable packaging properties as compared with commercial PLA. The good mechanical strength of the material offers the potential application as a rigid packaging material and/or coating. The high stiffness of active PLA/kenaf composites offers potential applications in thin walled food containers, crates and food trays. Since the PLA/kenaf composites release thymol faster than neat PLA, as reflected in the AM activity results (*in vitro* and *in vivo*), the use of active PLA with the presence of kenaf filler for disposable food trays or containers is suggested. Moreover, based on the observations made during the investigation of the interaction and quantification of thymol in the active PLA and PLA/kenaf films as well as the AM activity of the materials, it is suggested that the polymer may protect the AM agent during storage while the presence of kenaf fibres may trigger the AM activity once the material exposed to a humid environment. In addition, only a slight decrease in the retained AM additive concentration was observed after storage suggesting that a sufficient amount of thymol remained in the polymeric matrix to enable these materials to remain active.

9.3 Recommendation for Further Research

It was found that the active PLA-based materials containing the natural additive thymol and a natural fibre filler are best suited for rigid packaging materials and ready-to-eat food products. Whilst achieving its original aims, the current study has opened up new avenues of enquiry that can be pursued in the future to possibly enable the commercial implementation of this or similar AM systems:

- The method of incorporating natural fibres and thymol into PLA during melt blending and/or extrusion processing could be varied in order to further study the interaction and quantification of thymol in the system. One suggestion is to dope the fibres with thymol solutions prior to processing *via* melt mixing with PLA.

- The effect of nano-fillers derived from kenaf fibres on the mechanical, thermal and AM activity of PLA as well as the potential of these particles to migrate into food products could be investigated to see if there are further advantages to be had by nano-sizing the filler in these systems.
- The effect of microencapsulation on the volatility of the AM agent during processing could be investigated as a possible way of optimizing the retention of the AM agent in the system prior to use as an AM packaging material.
- The potential for incorporating other AM agents such as immobilized AM substances onto PLA-based materials containing natural fibres as well as the inhibitory effect of thymol against other spoilage or pathogenic microorganisms on various foodstuffs could be investigated in other major studies.
- The lifetime of the active PLA composites could be further investigated with studies such as the effect of aging on the films and composites, the effect of molecular weight changes over time, the effect of food contact on the properties of materials, the effect of soil-based or model landfill degradation, as well as a full life cycle assessment of the materials including the impact of increasing food preservation due to the addition of the AM additives.

Appendix A: Properties of Polymer and AM Agent

Table A. 1 Properties of poly(lactic acid)

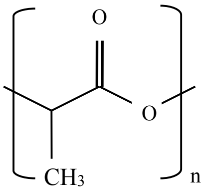
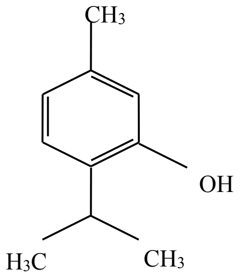
	Poly(lactic acid)
Product Code	7001D Ingeo^{TM} Injection Stretch Blow Moulding Bottle Grade
Company	NatureWorks LLC, USA
Structure	
Molecular Formula	$(C_3H_4O_2)n$
Specific Gravity	1.24
Melt Flow Rate	6 g/10 min (210°C, 2.16kg)
Relative Viscosity	3.9-4.1
Crystalline Melt Temperature	145-155°C
Glass Transition Temperature	52-58°C
Crystallization Temperature	100-120°C
Transmission Rates	Oxygen 550 cc-mil/m^2/24 hr-atm
	Carbon Dioxide 3000 cc-mil/m^2/24 hr-atm
	Water Vapour 325 g-mil/m^2/24 hr-atm
Clarity	Transparent
Tensile Yield Strength	60 MPa
Tensile Strength at Break	53 MPa
Tensile Modulus	3.6 GPa
Tensile Elongation	6%
Notched Impact Strength	16 J/m
Flexural Strength	83 MPa
Flexural Modulus	3.8 GPa
Heat Distortion Temperature	55°C

Table A. 2 Properties of thymol

	Thymol
Product Code	T0501
Assay	≥99.5%
Company	Sigma-Aldrich Pty, Ltd.
Synonyms	2-Isopropyl-5-methlphenol; 5-Methyl-2-isopropylphenol; 5-Methyl-2-(1-methylethyl) phenol;
Structure	
Molecular Formula	$C_{10}H_{14}O$
Molecular Weight	150.22 g/mol
CAS Number	89-83-8
Appearance/Physical State	Crystalline and colourless
pH	7 at 1 g/l
Density	0.965 g/cm^3 at 25°C
Melting Point	48-51°C
Boiling Point	232°C
Flash Point	110°C
Solubility	Slightly soluble in water and glycerol, very soluble in alcohol and in ether, freely soluble in essential oils and in fatty oils

Appendix B: Supplementary Figures Pertaining to the Properties of the PLA-based Materials

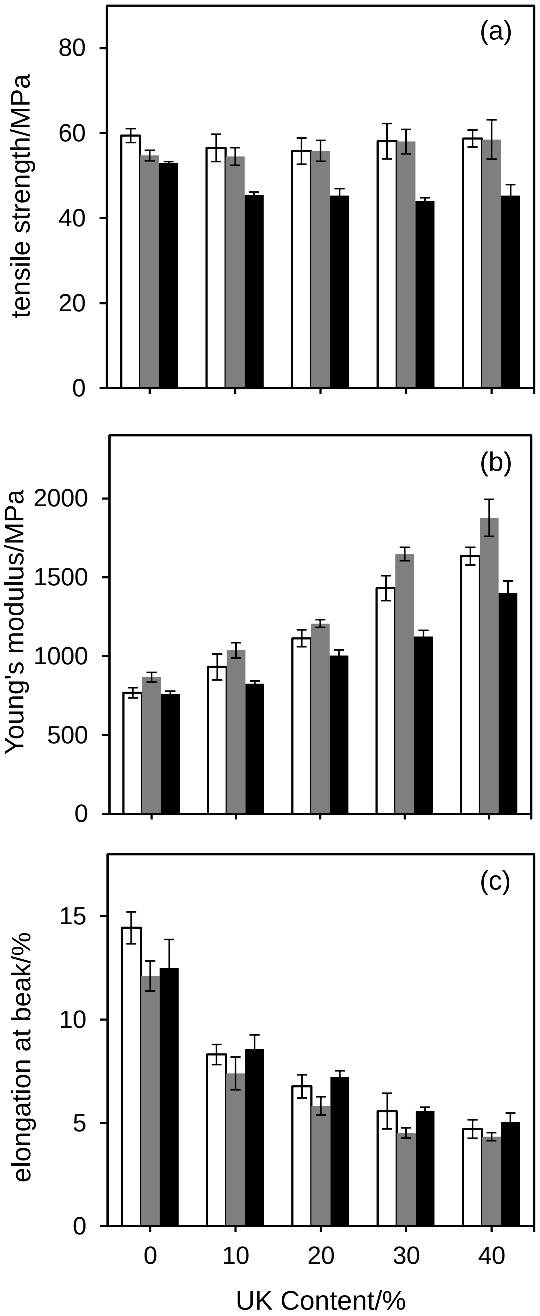


Figure B-1. Tensile properties of PLA/untreated kenaf composites containing: (□) zero, (■) 5% (w/w) and (■) 10% (w/w) thymol: (a) tensile strength; (b) Young's modulus and (c) percent elongation at break

Appendix C: Supplementary Figures Pertaining to the Migration of Thymol into Food Simulants

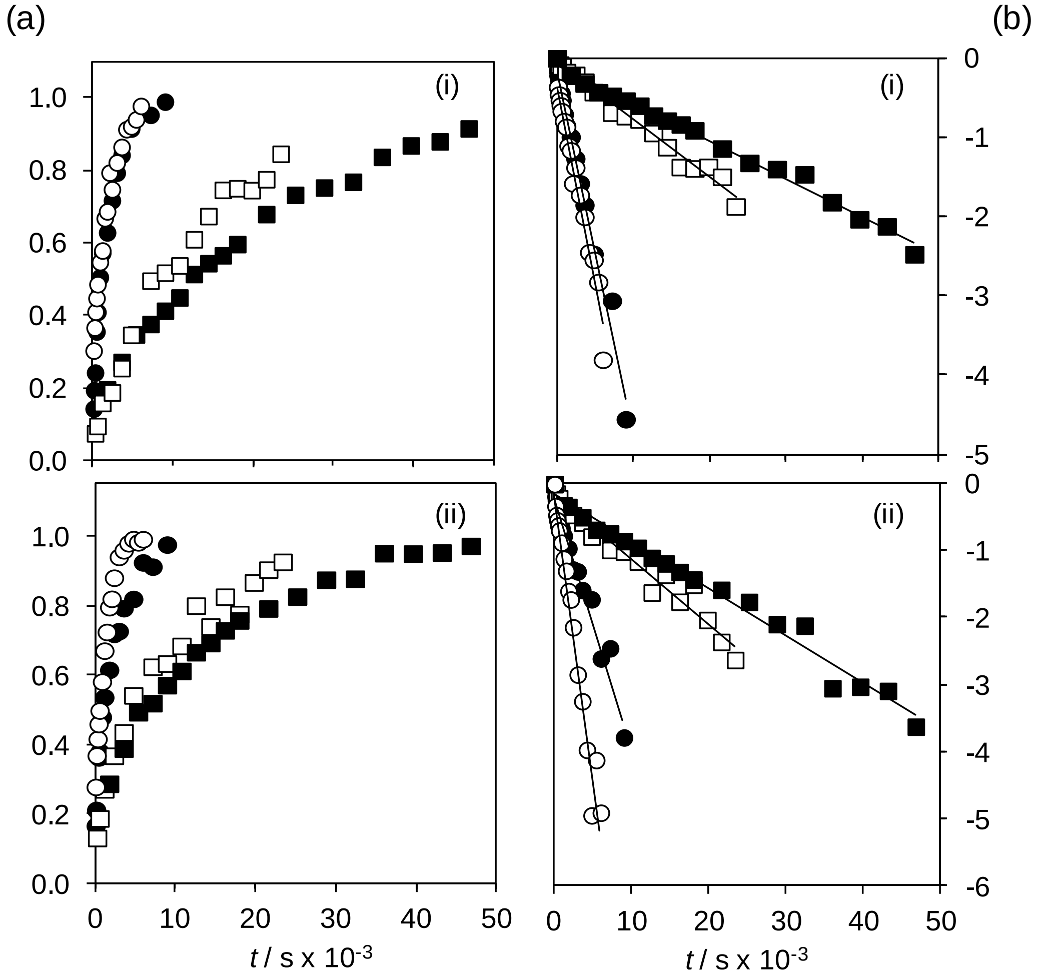


Figure C-1. Plot of (a) the mass fraction m_t/m_∞ *versus t* and (b) $\ln(1 - m_t/m_\infty)$ *versus t* for the release of thymol from (i) PLA and (ii) PLA/kenaf films into 150 mL/L ethanol/water at: ■ 83°C, □ 75°C, ● 65°C and ○ 60°C where m_t is the mass of thymol released from the film at time *t* and m_∞ is the amount of thymol released from the film at equilibrium ($t = \infty$).

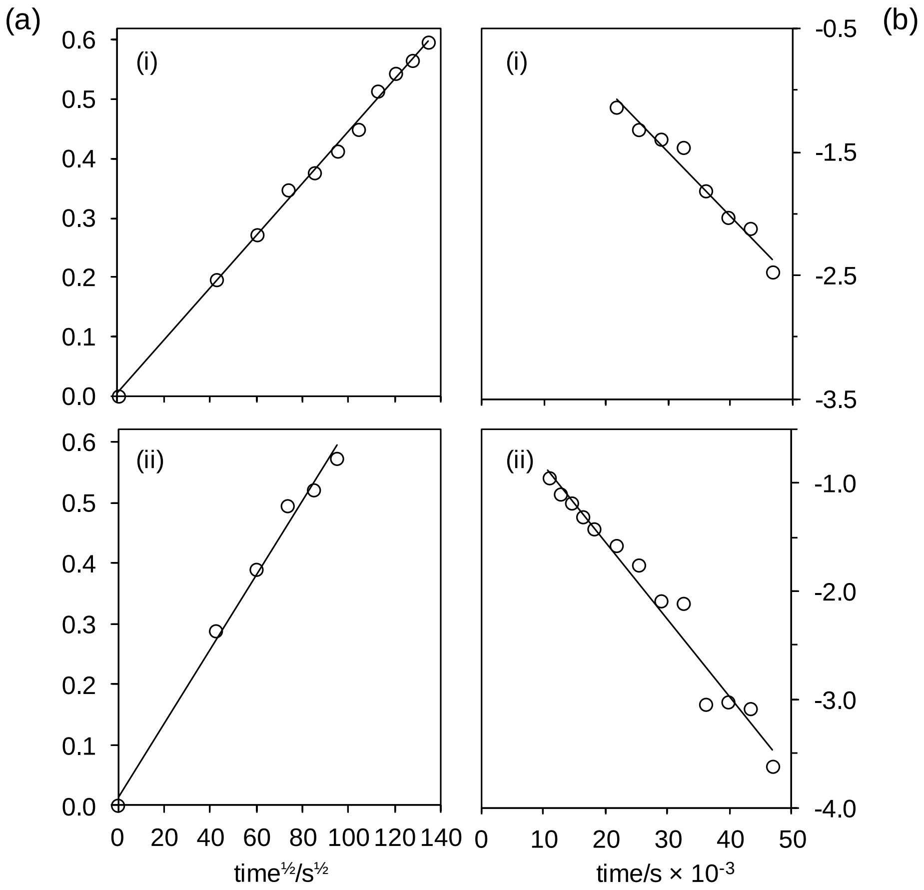


Figure C-2. Plot of (a) mass fraction m_t/m_∞ *versus* $t^{1/2}$and (b) ln(1 - m_t/m_∞) *versus* t for the release of thymol from (i) PLA and (ii) PLA/kenaf films into 150 mL/L ethanol/water at 60°C where m_t is the mass of thymol released from the film at time t and m_∞ is the amount of thymol released from the film at equilibrium ($t = \infty$).

Appendix D: Supplementary Figures Pertaining to Antimicrobial Activity of PLA-based Materials containing Thymol on Solid and Liquid Media

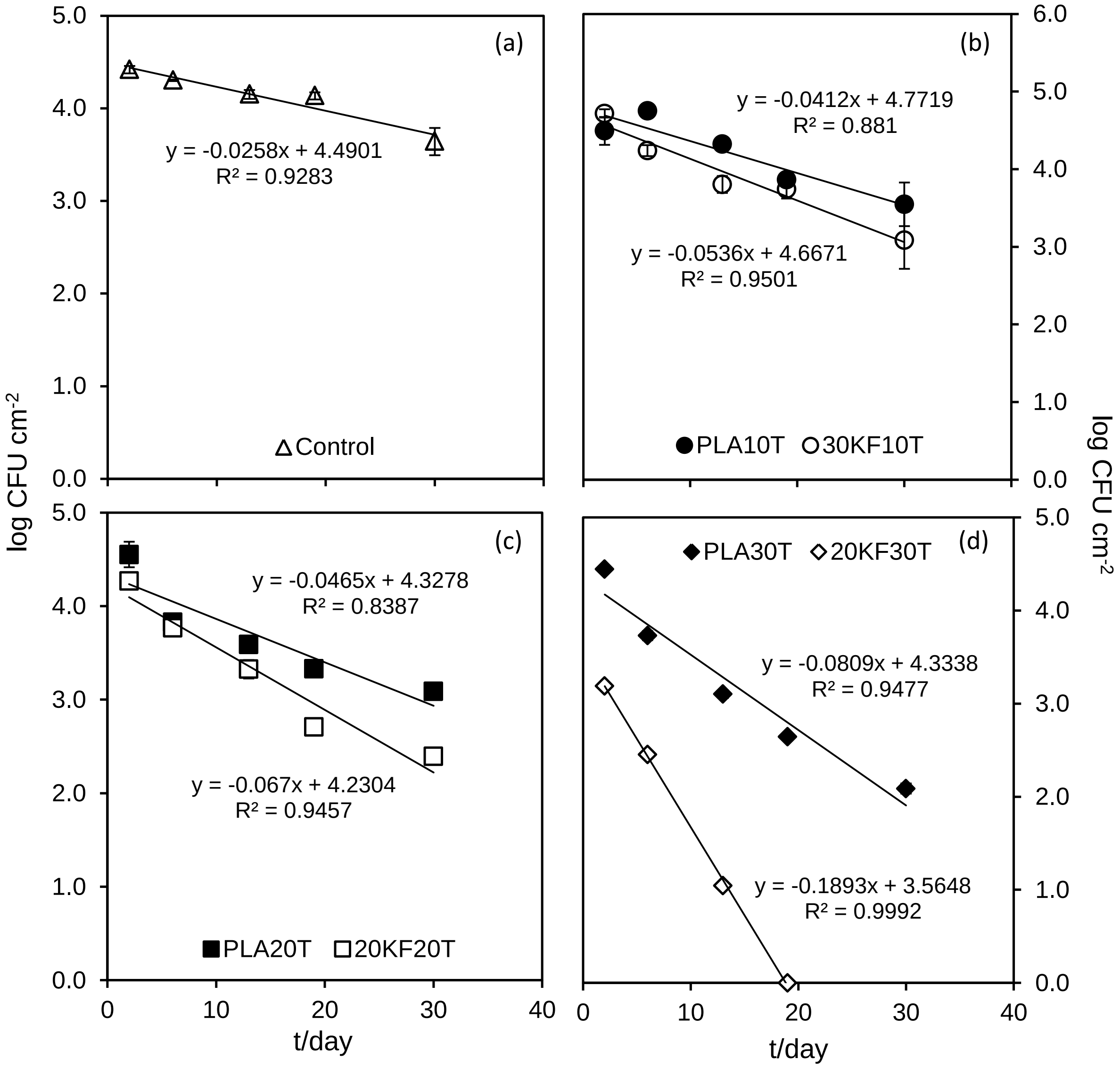


Figure D-1. Plot of bacteria count of log CFU cm^{-2} versus day of the AM activity on meat samples against *E. coli* for: (a) control without film, (b) PLA films containing 10% w/w thymol, (c) PLA films containing 20% w/w thymol and (d) PLA films containing 30% w/w thymol.

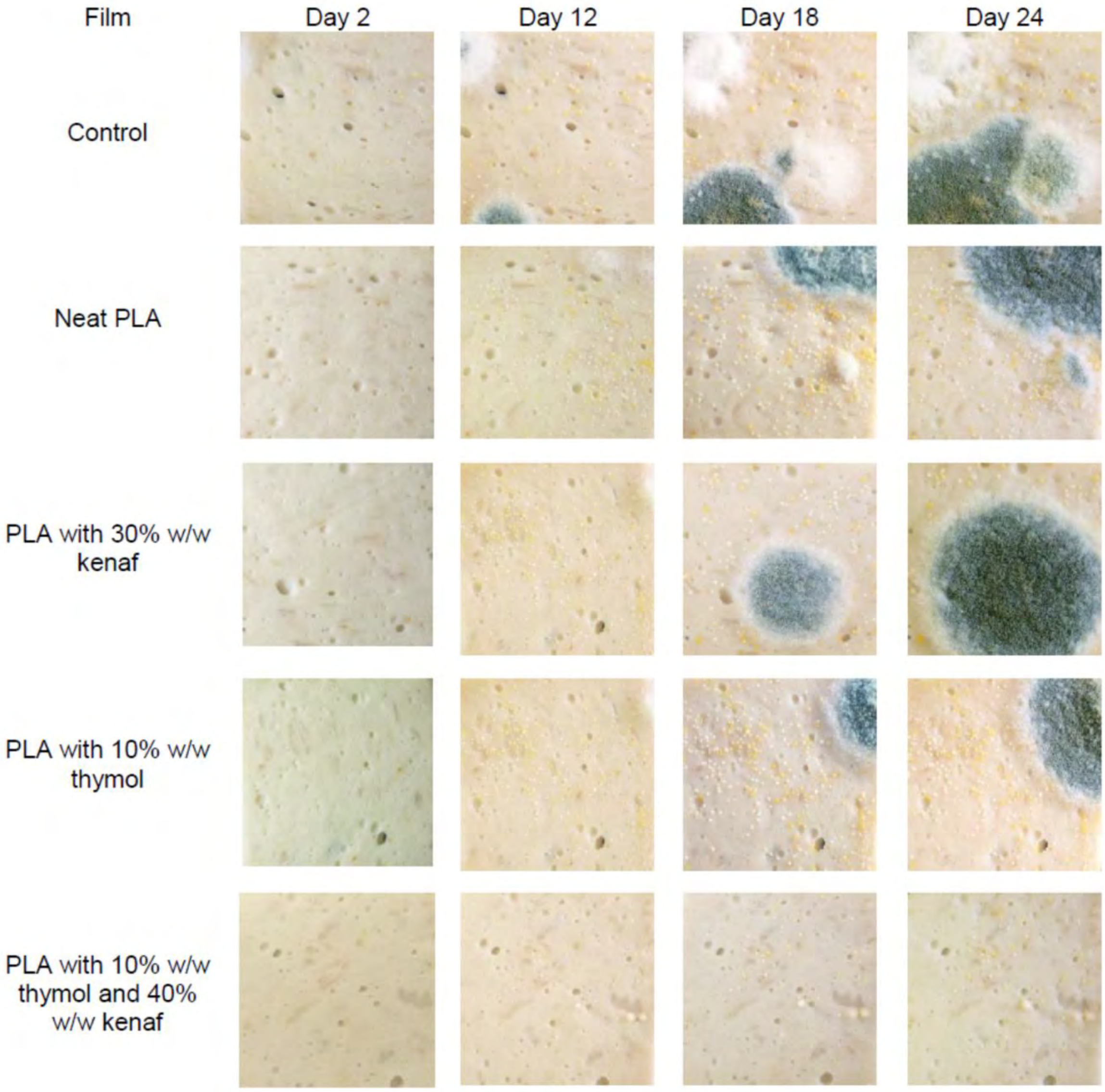


Figure D-2. Fungal coverage on the surface of meat samples store at 10°C for the control, neat PLA, PLA film containing 30% w/w kenaf, PLA film containing 10% w/w thymol and PLA/kenaf film containing 10% w/w thymol and 40% w/w kenaf fibres.

Figure D-3. Antimicrobial effect of films against *E. coli* after 24h of incubation at 37°C of (a) PLA film containing 20% w/w thymol and (d) PLA/kenaf film containing 20% w/w thymol and 20% w/w kenaf fibres.

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